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(54) Title: ENTEROCOCCUS ANTIGENS

(57) Abstract: The present invention discloses isolated nucleic acid molecules encoding a hyperimmune serum reactive antigen or a fragment thereof as well as hyperimmune serum reactive antigens or fragments thereof from *E.faecalis*, methods for isolating such antigens and specific uses thereof.

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ENTEROCOCCUS ANTIGENS

The present invention relates to isolated nucleic acid molecules, which encode antigens for *Enterococci*, e.g. *Enterococci faecalis*, which are suitable for use in preparation of pharmaceutical medicaments for the prevention and treatment of bacterial infections caused by *Enterococci*.

Enterococci are gram-positive bacteria that are normal inhabitants of the alimentary tract of humans and animals. They have been recognized as a cause of infective endocarditis for more than a century {Murray, B., 1990} and have gained prominence over the last two decades as being among the most common pathogens found in hospital-acquired infections, and surgical-site infections {Richards, M. et al., 2000}. The increasing importance of enterococci as nosocomial pathogens can be attributed in part to intrinsic and acquired antibiotic resistance {Murray, B., 1990}; {Rice, L., 2001}. Treatment of multi-drug-resistant enterococcal infections poses a significant challenge to clinicians {Cetinkaya, Y. et al., 2000}; {Gold, H., 2001}, and the potential of these organisms to serve as a reservoir for antibiotic resistance genes is of great concern {French, G., 1998}; {Noble, W. et al., 1992}; {Poyart, C. et al., 1997}.

The classification of enterococci as group D streptococci dates back to the early 1930's. In 1984, enterococci were given formal genus status after several studies demonstrated a more distant relationship with the streptococci.

Enterococci are generally considered commensals of the gastrointestinal tract of a variety of organisms including man. Although there are more than 14 different species of enterococci, *E. faecalis* and *E. faecium* are the species most commonly found in humans. Several intrinsic features of *Enterococcus* may allow members of this genus to survive for extended periods of time, leading to its persistence and nosocomial spread. The ability of enterococci to adapt and persist in the presence of detergents may allow them to survive inadequate cleaning regimens, contributing to their persistence in the hospital. The intrinsic ruggedness of enterococci also confers an unusual level of tolerance to several classes of antibiotics including aminoglycosides, -lactams and quinolones. For example, the resistance of enterococci to aminoglycosides results from the ability of enterococci to block the uptake of the drug at the cell wall. Although the mechanism of high-level resistance was determined to be the result of a bifunctional enzyme {Ferretti, J. et al., 1986}, the molecular basis for the intrinsic resistance of enterococci to low-levels of aminoglycosides remains to be determined.

Among the Enterococci, *E. faecium* is unique because it is commonly used in production of fermented foods, and is also used as a probiotic bacterium. In recent years, *E. faecium* has been less acceptable as a food fermentation organism because of concern that this bacterium may be an intermediate host for spreading of antibiotic resistance to bacteria involved in human infections. Despite these concerns, *E. faecium* is still amongst the most common bacteria found in foods fermented by lactic acid bacteria. Many isolates of *E. faecium* have been shown to produce bacteriocins (antimicrobial peptides) that are able to kill or inhibit growth of pathogens such as listeria, clostridia, bacilli, and staphylococci. Such bacteriocins may contribute to the preservative effect of food fermentations, and is one reason why they have been chosen as starter cultures in the production of fermented food. Recently, enterocins have been implemented successfully in treatment of mastitis in cattle.

Besides the applications for food production, as probiotics and in treatment of animal disease, and more importantly, enterococci are emerging opportunistic human pathogens. This is due to their intrinsic pathogenic potential, and, even more because of their ability to rapidly acquire antibiotic resistance genes. *E. faecium* and *E. faecalis* are the causing agents of a large percentage of hospital-acquired infections, including superinfections.

Enterococci normally colonize the gastrointestinal tract of man. They are found in relative abundance in human feces. A close association is likely to exist between enterococci and their host, or the organism would be eliminated due to normal intestinal motility. Many infection-derived enterococcal isolates were found to be clonal, indicating nosocomial transmission. Moreover, a number of studies have documented patient colonization following hospital admission, and have shown that colonization with multiple resistant strains is a predisposing factor for subsequent infection.

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One of the enigmas of nosocomial enterococcal infection not easily explained is the ready colonization of an ecology already occupied by members of the same species. As noted, antibiotics lacking substantial anti-enterococcal activity (i.e. antibiotics that do not deleteriously affect indigenous enterococci) are important predisposing factors for infection. These infections are frequently caused by multiple resistant enterococcal isolates that have been exogenously acquired and appear to have out competed indigenous enterococci in the absence of direct selection.

The fact that exogenous, multiple resistant, nosocomially transmitted enterococci efficiently colonize the gastrointestinal tract suggests that they may not compete directly for the same niche as indigenous strains.

Infection caused by the genus *Enterococcus* include a) bacteremia, b) urinary tract infections c) endophthalmitis, d) endocarditis and also wound and intra-abdominal infections. Approximately $\frac{3}{4}$ of the infections are caused by the species *E. faecalis*, the rest by *E. faecium*.

a) bacteremia

Nosocomial surveillance data for the period October 1986-April 1997 list enterococci as the third most common cause of nosocomial bacteremia, accounting for 12.8% of all isolates. The translocation of enterococci across an intact intestinal epithelial barrier is thought to lead to many bacteremias with no identifiable source. The risk factors for mortality associated with enterococcal bacteremia include severity of illness, patient age, and use of broad spectrum antibiotics, such as third-generation cephalosporins or metronidazole. These studies suggest that high-level aminoglycoside resistance does not affect clinical outcome, and that the presence of the *E. faecalis* cytolysin (hemolysin) may enhance the severity of the infection.

b) urinary tract infections

Enterococci have been estimated to account for 110,000 urinary tract infections (UTI) annually in the United States. A few studies have been aimed at understanding the interaction of enterococci with uroepithelial tissue. A potential role for the plasmid-encoded aggregation substance in the adhesion of enterococci to renal epithelial cells has been demonstrated. *E. faecalis* harboring the pheromone responsive plasmid pAD1, or various isogenic derivatives, were better able to bind to the cultured pig renal tubular cell line, LLC-PK, than plasmid free cells. Their findings also showed that a synthetic peptide containing the fibronectin motif, Arg-Gly-Asp-Ser, could inhibit binding. This structural motif mediates the interaction between fibronectin and eucaryotic surface receptors of the integrin family.

c) endophthalmitis

Colonization of host tissue may play a role in the pathogenesis of endophthalmitis. Enterococci are among the most destructive agents that cause this post-operative complication of cataract surgery. Experiments designed to determine whether aggregation substance targeted *E. faecalis* to alternate anatomical structures within the eye showed that enterococci attach to membranous structures in the vitreous, but that such adherence is not dependent on the presence of aggregation substance.

d) endocarditis

Of the diverse infections caused by enterococci, infective endocarditis (IE) is one of the most therapeutically challenging. Enterococci are the third leading cause of infective endocarditis, accounting for 5-20% of cases of native valve IE, and 6-7% of prosthetic valve endocarditis. The presence of the pheromone-responsive plasmid pAD1 enhances vegetation formation in enterococcal endocarditis. Serum from a patient with *E. faecalis* endocarditis was used to identify an antigen selectively expressed in serum but not in broth culture [Lowe, A. et al., 1995]. This protein antigen, designated EfaA, has extensive sequence similarity with several streptococcal adhesions and might function as an important adhesin in endocarditis.

Ampicillin is the therapy of choice for enterococcal infections. For serious enterococcal infection, particularly for endocarditis, aminoglycosides are critical as part of combination therapy with penicillin or ampicillin. Although enterococci are intrinsically resistant to low levels of aminoglycosides, the addition of the cell wall inhibitors to aminoglycoside will result in an enhanced killing by the synergistic

action of the two antimicrobials. With the increasing incidence of high level resistance to aminoglycosides and penicillins, vancomycin has become the only choice available for the treatment of enterococcal infections. Then, vancomycin resistance was reported in clinical isolates of enterococci in 1988, followed by an outbreak caused by vancomycin-resistant enterococci (VRE). In U.S. hospitals the percentage of nosocomial enterococci resistant to vancomycin increased from 0.3% in 1989 to 7.9% in 1993. Among patients in intensive care units with nosocomial infections an increase was even more dramatic; from 0.4% in 1989 to 13.6% in 1993, a 34-fold increase in the 4-year period.

In addition to a higher mortality rate, vancomycin-resistant enterococcal infections cost on average about \$25,000 more to treat and doubled the patients' length of stay in the hospital.

The dramatic increase in vancomycin resistance, especially among *E. faecium* isolates, indicates that enterococcal infection will pose an increasing challenge in the future. An obvious therapeutic alternative is vaccination with the aim to induce protective immune responses, which prevents or attenuates infections.

Vaccine development is hindered by the lack of sufficient knowledge about the elements of protective immunity against enterococcal infections. There are reports that neutrophil mediated killing of enterococci was largely a function of complement with antibody playing a less essential but potentially important role, though additional evidence for the importance of anti-enterococcal antibodies in promoting clearance by opsonophagocytic killing was recently reported [Gagliani, M. et al., 1997].

The importance of surface proteins in human immunity to *Enterococcus* already has been appreciated. It is apparent that all clinical isolates express surface proteins with activity relevant to host immune defense. The enterococcal surface protein (Esp) [Shankar, V. et al., 1999], gelatinase, cytolysin [Haas, W. et al., 2002] and aggregation substance (AS) surface protein [Sussmuth, S. et al., 2000] are well-characterized biochemically and genetically, and have also been shown to be immunogenic [Xu, Y. et al., 1997]. In an animal model of infective endocarditis specific antibodies against the aggregation substance were still not protective [McCormick, J. et al., 2001].

Thus, there remains a need for an effective treatment to prevent or ameliorate enterococcal infections. Vaccines capable of showing cross-protection against the majority of *Enterococcus* strains causing human infections could also be useful to prevent or ameliorate infections caused by all other enterococcal species, namely *E. faecalis* and *E. faecium*.

A vaccine can contain a whole variety of different antigens. Examples of antigens are whole-killed or attenuated organisms, subfractions of these organisms/tissues, proteins, or, in their most simple form, peptides. Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T-cells (CTL) recognize antigens in form of short usually 8-11 amino acids long peptides in conjunction with major histocompatibility complex (MHC). B-cells can recognize linear epitopes as short as 4-5 amino acids, as well as three-dimensional structures (conformational epitopes). In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system necessary. Primarily, adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APCs). These cells usually first encounter the antigen(s) followed by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response. The adjuvant may also locally retain antigens and co-injected other factors. In addition the adjuvant may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

Currently vaccines against enterococcal infection are only in the research stages of development. Efforts are focused not only on capsular polysaccharide (CPS) as immunogens [Huebner, J. et al., 2000], but also on virulence factors and membrane/surface proteins.

The development of protein conjugated vaccines are no doubt a great new addition to the armamentarium

in the battle against enterococcal infections, but the vaccine can contain only a limited number of enterococcal proteins and given adequate ecological pressure, variation of the pathogenicity island and plasmids by non-vaccine clinical isolates remains a real threat. Moreover polysaccharide antigens used for active immunization do not provide immunological memory in humans. Conjugation of CPS to non-enterococcal related immunogenic protein carriers (e.g. tetanus toxoid, cholera toxin B subunit, etc.) has been shown to be beneficial in inducing higher concentrations of antibodies in vaccines, but it does not provide pathogen-specific B cell and T cell epitopes which would recruit memory B and T cells during a real infection to support the most effective host response. To be able to supplement the enterococcal vaccines with proteins fulfilling these criteria it is necessary to identify conserved immunogenic enterococcal-specific surface proteins.

There is a great potential for passive antibody-based therapy. There have been already attempts to use human intravenous immunoglobulin (IVIG) preparations for prevention. Recent advances in the technology of monoclonal antibody production provide the means to generate human antibody reagents and reintroduce antibody therapies, while avoiding the toxicities associated with serum therapy. Immunoglobulins are an extremely versatile class of antimicrobial proteins that can be used to prevent and treat emerging infectious diseases. Antibody therapy has been effective against a variety of diverse microorganisms reviewed in [Burnie, J. et al., 1998]. Anti-enterococcal mAb could be given therapeutically to immunosuppressed patient, due to organ transplantation, cancer, HIV infection and other causes.

Certain proteins or enzymes displayed on the surface of gram-positive organisms significantly contribute to pathogenesis, are involved in the disease process caused by these pathogens. Often, these proteins are involved in direct interactions with host tissues or in concealing the bacterial surface from the host defense mechanisms [Navarre, W. et al., 1999]. *E. faecalis* is not an exception in this regard. Several surface proteins are characterized as virulence factors, important for enterococcal pathogenicity reviewed in [Jett, B. et al., 1994]. If antibodies to these proteins could offer better protection to humans than polysaccharides, they could provide the source of a novel, protein-based enterococcal vaccine to be used in conjunction with or in place of the more traditional capsular polysaccharide vaccine. The use of some of the above-described proteins as antigens for a potential vaccine as well as a number of additional candidates resulted mainly from a selection based on easiness of identification or chance of availability. There is a demand to identify relevant antigens for *E. faecalis* in a more comprehensive way.

The present inventors have developed a method for identification, isolation and production of hyperimmune serum reactive antigens from a specific pathogen, especially from *Staphylococcus aureus* and *Staphylococcus epidermidis* (WO 02/059148). However, given the differences in biological property, pathogenic function and genetic background, *Enterococcus faecalis* is distinctive from *Staphylococcus* strains. In order to identify relevant serum sources three major types of human sera were collected from healthy adults, as well from patients with enterococcal infections and naïve individuals, young children between 5 and 10 months of age, after they already lost maternal antibodies (as negative controls). A large percentage of individuals are exposed to enterococci in the environment that can induce antibodies in the host. Disease, which mainly occurs in hospitals, might be associated with low levels of specific antibodies against Enterococci, and consequently less efficient phagocytic elimination. To select for appropriate screening reagents, a series of immunoassays (mainly ELISA and immunoblotting) were performed with bacterial lysate and culture supernatant proteins to measure anti-*E. faecalis* IgG antibody levels. Sera from high titer individuals were included in the genomic-based antigen identification.

The genomes of the two bacterial species *E. faecalis* and *S. aureus* by itself show a number of important differences. The genome of *E. faecalis* contains app. 3.22 Mb, while *S. aureus* harbours 2.85 Mb. They have an average GC content of 37.5 and 33%, respectively and approximately 1/3 of the encoded genes are not shared between the two pathogens. In addition, the two bacterial species require different growth conditions and media for propagation. A list of the most important diseases, which can be inflicted by the

two pathogens is presented below. *S. aureus* causes mainly nosocomial, opportunistic infections: impetigo, folliculitis, abscesses, boils, infected lacerations, endocarditis, meningitis, septic arthritis, pneumonia, osteomyelitis, scalded skin syndrome (SSS), toxic shock syndrome. *E. faecalis* causes mainly infections which are not highly toxigenic, highly invasive, or highly infectious by most measures. They do, nevertheless, cause a substantial amount of human disease such as bacteremia, urinary tract infections, endocarditis and intra-abdominal infections.

The complete genome sequence of *E. faecalis* V583, a vancomycin-resistant clinical isolate, was determined by the random shotgun sequencing strategy (GenBank accession number for chromosome and the plasmids are as follows: AE016830 (chromosome), AE016833 (pTEF1), AE016831 (pTEF2), AE016832 (pTEF3)); see www.tigr.org/tigrscripts/CMR2/CMRHomePage.spl. [Paulsen, I. et al., 2003].

The complete genome of *E. faecium* strain DO (ATCC BAA-472, TEX16, TX0016) with an estimated genome size of 2.8 Mbp has been sequenced. The genome is currently being computed and annotated by the Baylor College of Medicine's Human Genome Sequencing Center and the University of Texas Center for the Study for Emerging and Re-emerging Pathogens (CSERP); see: www.hgsc.bcm.tmc.edu/microbial/Efaecium/

The problem underlying the present invention was to provide means for the development of medicaments such as vaccines against *E. faecalis* infection. More particularly, the problem was to provide an efficient, relevant and comprehensive set of nucleic acid molecules or hyperimmune serum reactive antigens from *E. faecalis* that can be used for the manufacture of said medicaments.

Therefore, the present invention provides an isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence which is selected from the group consisting of:

- a) a nucleic acid molecule having at least 70% sequence identity to a nucleic acid molecule selected from Seq ID No 1-2, 4-8, 10, 12-18, 20-23, 25-26, 29-43, 45-62, 64-74, 76-77, 79-83, 85-89, 91-92, 94-114, 117-126, 128-146, 148-170, 373, 375, 379-381, 387, 392, 394, 397-399, 407-408, 410-411 and 415-424.
- b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
- c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
- d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b), or c)
- e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid molecule defined in a), b), c) or d).

According to a preferred embodiment of the present invention the sequence identity is at least 80%, preferably at least 95%, especially 100%.

Furthermore, the present invention provides an isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of

- a) a nucleic acid molecule having at least 96%, preferably at least 98 %, especially 100 % sequence identity to a nucleic acid molecule selected from Seq ID No 3, 9, 11, 24, 27, 44, 63, 75, 84, 115-116, 127, 374, 376-378, 382-386, 388-391, 393, 395-396, 400-406, 409 and 412-414,
- b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
- c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
- d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b) or c),

- e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

According to another aspect, the present invention provides an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of

- a) a nucleic acid molecule selected from Seq ID No 90, 147.
- b) a nucleic acid molecule which is complementary to the nucleic acid of a),
- c) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).

Preferably, the nucleic acid molecule is DNA or RNA.

According to a preferred embodiment of the present invention, the nucleic acid molecule is isolated from a genomic DNA, especially from a *E. faecalis* genomic DNA.

According to the present invention a vector comprising a nucleic acid molecule according to any of the present invention is provided.

In a preferred embodiment the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens or fragments thereof encoded by the nucleic acid molecule according to the present invention.

The present invention also provides a host cell comprising the vector according to the present invention.

According to another aspect the present invention further provides a hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to the present invention.

In a preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of Seq ID No 171-172, 174-178, 180, 182-188, 190-193, 195-196, 199-213, 215-232, 234-244, 246-247, 249-253, 255-259, 261-262, 264-284, 287-296, 298-316, 318-340, 425, 427, 431-433, 439, 444, 446, 449-451, 459-460, 462-463 and 467-476.

In another preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of Seq ID No 173, 179, 181, 194, 197, 214, 233, 245, 254, 285-286, 297, 426, 428-430, 434-438, 440-443, 445, 447-448, 452-458, 461 and 464-466.

In a further preferred embodiment the amino acid sequence (polypeptide) is selected from the group consisting of Seq ID No 260, 317.

According to a further aspect the present invention provides fragments of hyperimmune serum-reactive antigens selected from the group consisting of peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region" of Table 1a and Table 1c; the serum reactive epitopes of Table 2, especially peptides comprising amino acids 4-10, 14-21, 30-36, 59-68, 77-82, 87-93, 96-105, 112-121, 125-133, 135-141, 150-162, 164-183, 192-203, 207-213, 215-226, 228-234, 241-247, 250-285, 302-308 and 135-148 of Seq ID No 171; 15-57, 60-73, 77-101, 108-134, 136-177, 185-201, 203-217, 226-240, 244-254, 272-277, 283-288, 292-343, 354-370, 380-398, 406-437, 439-453, 473-490, 532-538, 584-590, 595-601, 606-612, 664-677, 679-704, 715-724, 731-753, 759-772, 786-794, 814-862 and 657-684 of Seq ID No 172; 4-9, 15-36, 41-47, 54-60, 75-81, 114-120, 131-146, 152-158, 174-182, 194-202, 208-215, 218-226, 255-271, 276-285, 290-295, 302-311, 318-328, 330-344, 352-359, 365-377, 388-395, 398-405, 426-432, 439-449, 455-500, 505-513, 531-537, 542-552, 554-561, 587-595, 606-612, 718-734, 763-771, 775-782, 792-801, 805-812, 822-828, 830-843, 849-863, 876-894, 905-911, 919-926, 935-947, 949-958, 968-979, 1009-1016, 1029-1045, 1047-

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82-94, 111-118, 125-131, 206-212, 261-266, 310-320, 328-338, 345-351, 353-360, 414-420, 424-434, 440-447, 451-500, 506-516, 548-561, 566-572, 584-591, 601-622, 630-636, 650-659, 661-667, 674-699, 703-711, 717-729, 736-744, 752-759, 765-771, 813-822, 826-842, 852-868, 870-877, 881-895, 897-906, 913-922 and 602-671 of Seq ID No 452; 12-18, 20-25, 43-54, 56-65, 73-79, 82-88, 99-111, 136-142, 153-169, 171-183, 195-223, 229-248, 255-260, 272-277, 281-292, 298-319, 322-329, 332-351, 363-379, 381-389 and 275-304 of Seq ID No 453; 4-9, 34-48, 65-77, 101-106, 111-131, 138-153, 186-191, 230-250 and 148-219 of Seq ID No 454; 4-23, 30-35, 42-53, 67-76, 82-87, 101-108, 112-130, 132-138, 147-152, 161-183, 187-208, 218-225, 265-283, 295-303, 306-317, 322-334, 338-357, 360-368, 370-383, 387-398, 400-419, 421-430, 104-182 and 240-304 of Seq ID No 455; 4-12, 63-69, 94-102, 146-164, 166-173, 175-181, 193-207, 263-281, 286-295, 301-306, 330-343, 369-378, 382-388, 414-420, 422-430, 438-454, 456-462, 472-531, 543-560, 581-591, 596-605, 614-623, 626-635, 656-662, 669-676, 683-690, 693-698, 705-711, 728-736, 752-764 and 69-102 of Seq ID No 456; 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182, 187-196, 206-218, 224-242, 248-253, 258-277 and 184-253 of Seq ID No 464; 10-17, 56-67, 72-82, 94-99, 106-113, 166-173, 229-235, 243-283, 295-301, 313-321, 326-331, 342-348, 396-414, 423-435, 446-452, 454-462, 496-502, 511-534, 543-556, 563-570, 586-593, 616-626, 638-645, 653-662, 679-696, 731-737, 766-774, 776-782, 790-796, 810-817, 825-835, 837-846 and 540-615 of Seq ID No 465; 13-24, 30-36, 73-81, 89-95, 109-115, 117-143, 161-173, 179-189, 226-244, 251-261, 275-281, 298-305, 307-315, 323-328, 364-374, 69-186 and 264-354 of Seq ID No 466; 19-25 and 6-22 of Seq ID No 467; 6-39, 59-68 and 43-62 of Seq ID No 468; 6-14, 22-32 and 1-27 of Seq ID No 469; 4-41 and 28-40 of Seq ID No 470; 8-14 and 4-19 of Seq ID No 471; 4-10, 12-22, 30-35 and 6-33 of Seq ID No 472; 4-16, 24-33 and 37-54 of Seq ID No 473; 2-23 of Seq ID No 474; 4-21, 27-33, 36-41 and 14-34 of Seq ID No 475; 4-14, 24-30, 37-42, 57-78, 83-89, 94-103, 113-131 and 100-122 of Seq ID No 476.

The present invention also provides a process for producing a *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof according to the present invention comprising expressing one or more of the nucleic acid molecules according to the present invention in a suitable expression system.

Moreover, the present invention provides a process for producing a cell, which expresses a *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof according to the present invention comprising transforming or transfecting a suitable host cell with the vector according to the present invention.

According to the present invention a pharmaceutical composition, especially a vaccine, comprising a hyperimmune serum-reactive antigen or a fragment thereof as defined in the present invention or a nucleic acid molecule as defined in the present invention is provided.

In a preferred embodiment the pharmaceutical composition further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), peptides containing at least two LysLeuLys motifs, especially κLKLK , neuroactive compounds, especially human growth hormone, alum, Freund's complete or incomplete adjuvants or combinations thereof.

In a more preferred embodiment the immunostimulatory substance is a combination of either a polycationic polymer and immunostimulatory deoxynucleotides or of a peptide containing at least two LysLeuLys motifs and immunostimulatory deoxynucleotides.

In a still more preferred embodiment the polycationic polymer is a polycationic peptide, especially polyarginine.

According to the present invention the use of a nucleic acid molecule according to the present invention or a hyperimmune serum-reactive antigen or fragment thereof according to the present invention for the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against enterococcal infection, is provided.

Also an antibody, or at least an effective part thereof, which binds at least to a selective part of the hyperimmune serum-reactive antigen or a fragment thereof according to the present invention is provided herewith.

In a preferred embodiment the antibody is a monoclonal antibody.

In another preferred embodiment the effective part of the antibody comprises Fab fragments.

In a further preferred embodiment the antibody is a chimeric antibody.

In a still preferred embodiment the antibody is a humanized antibody.

The present invention also provides a hybridoma cell line, which produces an antibody according to the present invention.

Moreover, the present invention provides a method for producing an antibody according to the present invention, characterized by the following steps:

- initiating an immune response in a non-human animal by administering an hyperimmune serum-reactive antigen or a fragment thereof, as defined in the invention, to said animal,
- removing an antibody containing body fluid from said animal, and
- producing the antibody by subjecting said antibody containing body fluid to further purification steps.

Accordingly, the present invention also provides a method for producing an antibody according to the present invention, characterized by the following steps:

- initiating an immune response in a non-human animal by administering an hyperimmune serum-reactive antigen or a fragment thereof, as defined in the present invention, to said animal,
- removing the spleen or spleen cells from said animal,
- producing hybridoma cells of said spleen or spleen cells,
- selecting and cloning hybridoma cells specific for said hyperimmune serum-reactive antigens or a fragment thereof,
- producing the antibody by cultivation of said cloned hybridoma cells and optionally further purification steps.

The antibodies provided or produced according to the above methods may be used for the preparation of a medicament for treating or preventing enterococcal infections.

According to another aspect the present invention provides an antagonist which binds to a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention.

Such an antagonist capable of binding to a hyperimmune serum-reactive antigen or fragment thereof according to the present invention may be identified by a method comprising the following steps:

- a) contacting an isolated or immobilized hyperimmune serum-reactive antigen or a fragment thereof according to the present invention with a candidate antagonist under conditions to permit binding of said candidate antagonist to said hyperimmune serum-reactive antigen or fragment, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said hyperimmune serum reactive antigen or fragment thereof; and
- b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the hyperimmune serum reactive antigen or the fragment thereof.

An antagonist capable of reducing or inhibiting the interaction activity of a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention to its interaction partner may be identified by a method comprising the following steps:

- a) providing a hyperimmune serum reactive antigen or a hyperimmune fragment thereof according to the present invention,
- b) providing an interaction partner to said hyperimmune serum reactive antigen or a fragment thereof, especially an antibody according to the present invention,
- c) allowing interaction of said hyperimmune serum reactive antigen or fragment thereof to said interaction partner to form an interaction complex,
- d) providing a candidate antagonist,

- e) allowing a competition reaction to occur between the candidate antagonist and the interaction complex,
- f) determining whether the candidate antagonist inhibits or reduces the interaction activities of the hyperimmune serum reactive antigen or the fragment thereof with the interaction partner.

The hyperimmune serum reactive antigens or fragments thereof according to the present invention may be used for the isolation and/or purification and/or identification of an interaction partner of said hyperimmune serum reactive antigen or fragment thereof.

The present invention also provides a process for *in vitro* diagnosing a disease related to expression of a hyperimmune serum-reactive antigen or a fragment thereof according to the present invention comprising determining the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen or fragment thereof according to the present invention or the presence of the hyperimmune serum reactive antigen or fragment thereof according to the present invention.

The present invention also provides a process for *in vitro* diagnosis of a bacterial infection, especially a enterococcal infection, comprising analyzing for the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen or fragment thereof according to the present invention or the presence of the hyperimmune serum reactive antigen or fragment thereof according to the present invention.

Moreover, the present invention provides the use of a hyperimmune serum reactive antigen or fragment thereof according to the present invention for the generation of a peptide binding to said hyperimmune serum reactive antigen or fragment thereof, wherein the peptide is an anticaline.

The present invention also provides the use of a hyperimmune serum-reactive antigen or fragment thereof according to the present invention for the manufacture of a functional nucleic acid, wherein the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.

The nucleic acid molecule according to the present invention may also be used for the manufacture of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.

The present invention advantageously provides an efficient, relevant and comprehensive set of isolated nucleic acid molecules and their encoded hyperimmune serum reactive antigens or fragments thereof identified from *E. faecalis* using an antibody preparation from multiple human plasma pools and surface expression libraries derived from the genome of *E. faecalis*. Thus, the present invention fulfils a widely felt demand for *E. faecalis* antigens, vaccines, diagnostics and products useful in procedures for preparing antibodies and for identifying compounds effective against enterococcal infections.

An effective vaccine should be composed of proteins or polypeptides, which are expressed by all strains and are able to induce high affinity, abundant antibodies against cell surface components of *E. faecalis*. The antibodies should be IgG1 and/or IgG3 for opsonization, and any IgG subtype and IgA for neutralisation of adherence and toxin action. A chemically defined vaccine must be definitely superior compared to a whole cell vaccine (attenuated or killed), since components of *E. faecalis*, which cross-react with human tissues or inhibit opsonization [Whitnack, E. et al., 1985] can be eliminated, and the individual proteins inducing protective antibodies and/or a protective immune response can be selected.

The approach, which has been employed for the present invention, is based on the interaction of enterococcal proteins or peptides with the antibodies present in human sera. The antibodies produced against *E. faecalis* by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity. In addition, the antigenic proteins as

identified by the bacterial surface display expression libraries using pools of pre-selected sera, are processed in a second and third round of screening by individual selected or generated sera. Thus the present invention supplies an efficient, relevant, comprehensive set of enterococcal antigens as a pharmaceutical composition, especially a vaccine preventing infection by *E. faecalis*.

In the antigen identification program for identifying a comprehensive set of antigens according to the present invention, at least two different bacterial surface expression libraries are screened with several serum pools or plasma fractions or other pooled antibody containing body fluids (antibody pools). The antibody pools are derived from a serum collection, which has been tested against antigenic compounds of *E. faecalis*, such as whole cell extracts and culture supernatant proteins. Preferably, 2 distinct serum collections are used: 1. With very stable antibody repertoire: normal adults, clinically healthy people, who are non-carriers and overcame previous encounters or currently carriers of *E. faecalis* without acute disease and symptoms, 2. With antibodies induced acutely by the presence of the pathogenic organism: patients with acute disease with different manifestations (e.g. *E. faecalis* endocarditis, urinary tract infection and bacteraemia). Sera have to react with multiple enterococci-specific antigens in order to be considered hyperimmune and therefore relevant in the screening method applied for the present invention. The antibodies produced against enterococci by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity.

The expression libraries as used in the present invention should allow expression of all potential antigens, e.g. derived from all surface proteins of *E. faecalis*. Bacterial surface display libraries will be represented by a recombinant library of a bacterial host displaying a (total) set of expressed peptide sequences of enterococci on a number of selected outer membrane proteins (LamB, BtuB, FhuA) at the bacterial host membrane (Georgiou, G., 1997); [Etz, H. et al., 2001]. One of the advantages of using recombinant expression libraries is that the identified hyperimmune serum-reactive antigens may be instantly produced by expression of the coding sequences of the screened and selected clones expressing the hyperimmune serum-reactive antigens without further recombinant DNA technology or cloning steps necessary.

The comprehensive set of antigens identified by the described program according to the present invention is analysed further by one or more additional rounds of screening. Therefore individual antibody preparations or antibodies generated against selected peptides which were identified as immunogenic are used. According to a preferred embodiment the individual antibody preparations for the second round of screening are derived from patients who have suffered from an acute infection with enterococci, especially from patients who show an antibody titer above a certain minimum level, for example an antibody titer being higher than 80 percentile, preferably higher than 90 percentile, especially higher than 95 percentile of the human (patient or healthy individual) sera tested. Using such high titer individual antibody preparations in the second screening round allows a very selective identification of the hyperimmune serum-reactive antigens and fragments thereof from *E. faecalis*.

Following the comprehensive screening procedure, the selected antigenic proteins, expressed as recombinant proteins or in vitro translated products, in case it can not be expressed in prokaryotic expression systems, or the identified antigenic peptides (produced synthetically) are tested in a second screening by a series of ELISA and Western blotting assays for the assessment of their immunogenicity with a large human serum collection (> 100 uninfected, > 50 patients sera).

It is important that the individual antibody preparations (which may also be the selected serum) allow a selective identification of the most promising candidates of all the hyperimmune serum-reactive antigens from all the promising candidates from the first round. Therefore, preferably at least 10 individual antibody preparations (i.e. antibody preparations (e.g. sera) from at least 10 different individuals having suffered from an infection to the chosen pathogen) should be used in identifying these antigens in the second screening round. Of course, it is possible to use also less than 10 individual preparations,

however, selectivity of the step may not be optimal with a low number of individual antibody preparations. On the other hand, if a given hyperimmune serum-reactive antigen (or an antigenic fragment thereof) is recognized by at least 10 individual antibody preparations, preferably at least 30, especially at least 50 individual antibody preparations, identification of the hyperimmune serum-reactive antigen is also selective enough for a proper identification. Hyperimmune serum-reactivity may of course be tested with as many individual preparations as possible (e.g. with more than 100 or even with more than 1,000).

Therefore, the relevant portion of the hyperimmune serum-reactive antibody preparations according to the method of the present invention should preferably be at least 10, more preferred at least 30, especially at least 50 individual antibody preparations. Alternatively (or in combination) hyperimmune serum-reactive antigens may preferably be also identified with at least 20%, preferably at least 30%, especially at least 40% of all individual antibody preparations used in the second screening round.

According to a preferred embodiment of the present invention, the sera from which the individual antibody preparations for the second round of screening are prepared (or which are used as antibody preparations), are selected by their titer against *E. faecalis* (e.g. against a preparation of this pathogen, such as a lysate, cell wall components and recombinant proteins). Preferably, some are selected with a total IgG titer above 10,000 U, especially above 12,000 U (U = units, calculated from the OD_{405nm} reading at a given dilution) when the whole organism (total lysate or whole cells) is used as antigen in the ELISA.

The antibodies produced against enterococci by the human immune system and present in human sera are indicative of the in vivo expression of the antigenic proteins and their immunogenicity. The recognition of linear epitopes by antibodies can be based on sequences as short as 4-5 amino acids. Of course it does not necessarily mean that these short peptides are capable of inducing the given antibody in vivo. For that reason the defined epitopes, polypeptides and proteins are further to be tested in animals (mainly in mice) for their capacity to induce antibodies against the selected proteins in vivo.

The preferred antigens are located on the cell surface or are secreted, and are therefore accessible extracellularly. Antibodies against cell wall proteins are expected to serve two purposes: to inhibit adhesion and to promote phagocytosis. Antibodies against secreted proteins are beneficial in neutralisation of their function as toxin or virulence component. It is also known that bacteria communicate with each other through secreted proteins. Neutralizing antibodies against these proteins will interrupt growth-promoting cross-talk between or within enterococcal species. Bioinformatic analyses (signal sequences, cell wall localisation signals, transmembrane domains) proved to be very useful in assessing cell surface localisation or secretion. The experimental approach includes the isolation of antibodies with the corresponding epitopes from human serum, and the generation of immune sera in mice against (poly)peptides selected by the bacterial surface display screens.

For that purpose, bacterial *E. coli* clones are directly injected into mice and immune sera are taken and tested in the relevant in vitro assay for functional opsonic or neutralizing antibodies. Alternatively, specific antibodies may be purified from human or mouse sera using peptides or proteins as substrate.

Host defence against *E. faecalis* relies mainly on innate immunological mechanisms. Inducing high affinity antibodies of the opsonic and neutralizing type by vaccination helps the innate immune system to eliminate bacteria and toxins. This makes the method according to the present invention an optimal tool for the identification of enterococcal antigenic proteins.

The skin and mucous membranes are formidable barriers against invasion by enterococci. However, once the skin or the mucous membranes are breached the first line of non-adaptive cellular defence begins its co-ordinate action through complement and phagocytes, especially the polymorphonuclear leukocytes (PMNs). These cells can be regarded as the cornerstones in eliminating invading bacteria. As enterococci

are primarily extracellular pathogens, the major anti-enterococcal adaptive response comes from the humoral arm of the immune system, and is mediated through three major mechanisms: promotion of opsonization, toxin neutralisation, and inhibition of adherence. It is believed that opsonization is especially important, because of its requirement for an effective phagocytosis. For efficient opsonization the microbial surface has to be coated with antibodies and complement factors for recognition by PMNs through receptors to the Fc fragment of the IgG molecule or to activated C3b. After opsonization, enterococci are phagocytosed and killed. Antibodies bound to specific antigens on the cell surface of bacteria serve as ligands for the attachment to PMNs and to promote phagocytosis. The very same antibodies bound to the adhesins and other cell surface proteins are expected to neutralize adhesion and prevent colonization. The selection of antigens as provided by the present invention is thus well suited to identify those that will lead to protection against infection in an animal model or in humans.

According to the antigen identification method used herein, the present invention can surprisingly provide a set of comprehensive novel nucleic acids and novel hyperimmune serum reactive antigens and fragments thereof of *E. faecalis*, among other things, as described below. According to one aspect, the invention particularly relates to the nucleotide sequences encoding hyperimmune serum reactive antigens which sequences are set forth in the Sequence listing Seq ID No 1-170, 373-424 and the corresponding encoded amino acid sequences representing hyperimmune serum reactive antigens are set forth in the Sequence Listing Seq ID No 171-340 and 425-476.

In a preferred embodiment of the present invention, a nucleic acid molecule is provided which exhibits 70% identity over their entire length to a nucleotide sequence set forth with Seq ID No 1-2, 4-8, 10, 12-18, 20-23, 25-26, 29-43, 45-62, 64-74, 76-77, 79-83, 85-89, 91-92, 94-114, 117-126, 128-146, 148-170, 373, 375, 379-381, 387, 392, 394, 397-399, 407-408, 410-411 and 415-424. Most highly preferred are nucleic acids that comprise a region that is at least 80% or at least 85% identical over their entire length to a nucleic acid molecule set forth with Seq ID No 1-2, 4-8, 10, 12-18, 20-23, 25-26, 29-43, 45-62, 64-74, 76-77, 79-83, 85-89, 91-92, 94-114, 117-126, 128-146, 148-170, 373, 375, 379-381, 387, 392, 394, 397-399, 407-408, 410-411 and 415-424. In this regard, nucleic acid molecules at least 90%, 91%, 92%, 93%, 94%, 95%, or 96% identical over their entire length to the same are particularly preferred. Furthermore, those with at least 97% are highly preferred, those with at least 98% and at least 99% are particularly highly preferred, with at least 99% or 99.5% being the more preferred, with 100% identity being especially preferred. Moreover, preferred embodiments in this respect are nucleic acids which encode hyperimmune serum reactive antigens or fragments thereof (polypeptides) which retain substantially the same biological function or activity as the mature polypeptide encoded by said nucleic acids set forth in the Seq ID No 1-2, 4-8, 10, 12-18, 20-23, 25-26, 29-43, 45-62, 64-74, 76-77, 79-83, 85-89, 91-92, 94-114, 117-126, 128-146, 148-170, 373, 375, 379-381, 387, 392, 394, 397-399, 407-408, 410-411 and 415-424.

Identity, as known in the art and used herein, is the relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. Identity can be readily calculated. While there exist a number of methods to measure identity between two polynucleotide or two polypeptide sequences, the term is well known to skilled artisans (e.g. *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG program package [Devereux, J. et al., 1984], BLASTP, BLASTN, and FASTA [Altschul, S. et al., 1990].

According to another aspect of the invention, nucleic acid molecules are provided which exhibit at least 96%, preferably at least 98 %, especially 100 % identity to the nucleic acid sequence set forth with Seq ID No 3, 9, 11, 24, 27, 44, 63, 75, 84, 115-116, 127, 374, 376-378, 382-386, 388-391, 393, 395-396, 400-406, 409 and

412-414.

According to a further aspect of the present invention, nucleic acid molecules are provided which are identical to the nucleic acid sequences set forth with Seq ID No 90, 147.

The nucleic acid molecules according to the present invention can as a second alternative also be a nucleic acid molecule which is at least essentially complementary to the nucleic acid described as the first alternative above. As used herein complementary means that a nucleic acid strand is base pairing via Watson-Crick base pairing with a second nucleic acid strand. Essentially complementary as used herein means that the base pairing is not occurring for all of the bases of the respective strands but leaves a certain number or percentage of the bases unpaired or wrongly paired. The percentage of correctly pairing bases is preferably at least 70 %, more preferably 80 %, even more preferably 90 % and most preferably any percentage higher than 90 %. It is to be noted that a percentage of 70 % matching bases is considered as homology and the hybridization having this extent of matching base pairs is considered as stringent. Hybridization conditions for this kind of stringent hybridization may be taken from Current Protocols in Molecular Biology (John Wiley and Sons, Inc., 1987). More particularly, the hybridization conditions can be as follows:

- Hybridization performed e.g. in 5 x SSPE, 5 x Denhardt's reagent, 0.1% SDS, 100 g/mL sheared DNA at 68°C
- Moderate stringency wash in 0.2xSSC, 0.1% SDS at 42°C
- High stringency wash in 0.1xSSC, 0.1% SDS at 68°C

Genomic DNA with a GC content of 50% has an approximate T_m of 96°C. For 1% mismatch, the T_m is reduced by approximately 1°C.

In addition, any of the further hybridization conditions described herein are in principle applicable as well.

Of course, all nucleic acid sequence molecules which encode the same polypeptide molecule as those identified by the present invention are encompassed by any disclosure of a given coding sequence, since the degeneracy of the genetic code is directly applicable to unambiguously determine all possible nucleic acid molecules which encode a given polypeptide molecule, even if the number of such degenerated nucleic acid molecules may be high. This is also applicable for fragments of a given polypeptide, as long as the fragments encode a polypeptide being suitable to be used in a vaccination connection, e.g. as an active or passive vaccine.

The nucleic acid molecule according to the present invention can as a third alternative also be a nucleic acid which comprises a stretch of at least 15 bases of the nucleic acid molecule according to the first and second alternative of the nucleic acid molecules according to the present invention as outlined above. Preferably, the bases form a contiguous stretch of bases. However, it is also within the scope of the present invention that the stretch consists of two or more moieties which are separated by a number of bases.

The present nucleic acids may preferably consist of at least 20, even more preferred at least 30, especially at least 50 contiguous bases from the sequences disclosed herein. The suitable length may easily be optimized due to the planned area of use (e.g. as (PCR) primers, probes, capture molecules (e.g. on a (DNA) chip), etc.). Preferred nucleic acid molecules contain at least a contiguous 15 base portion of one or more of the predicted immunogenic amino acid sequences listed in tables 1 and 2, especially the sequences of table 2 with scores of more than 10, preferably more than 20, especially with a score of more than 25. Specifically preferred are nucleic acids containing a contiguous portion of a DNA sequence of any sequence in the sequence protocol of the present application which shows 1 or more, preferably more

than 2, especially more than 5, non-identical nucleic acid residues compared to the published *Enterococcus faecalis* strain V583 genome (Paulsen, I. et al., 2003); GenBank accession AE016830 (chromosome), AE016833 (pTEF1), AE016831 (pTEF2), AE016832 (pTEF3), and/or any other published *E. faecalis* genome sequence or parts thereof. Specifically preferred non-identical nucleic acid residues are residues which lead to a non-identical amino acid residue. Preferably, the nucleic acid sequences encode for polypeptides having at least 1, preferably at least 2, preferably at least three different amino acid residues compared to the published *S.pyogenes* counterparts mentioned above. Also such isolated polypeptides, being fragments of the proteins (or the whole protein) mentioned herein e.g. in the sequence listing, having at least 6, 7, or 8 amino acid residues and being encoded by these nucleic acids are preferred.

The nucleic acid molecule according to the present invention can as a fourth alternative also be a nucleic acid molecule which anneals under stringent hybridisation conditions to any of the nucleic acids of the present invention according to the above outlined first, second, and third alternative. Stringent hybridisation conditions are typically those described herein.

Finally, the nucleic acid molecule according to the present invention can as a fifth alternative also be a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to any of the nucleic acid molecules according to any nucleic acid molecule of the present invention according to the first, second, third, and fourth alternative as outlined above. This kind of nucleic acid molecule refers to the fact that preferably the nucleic acids according to the present invention code for the hyperimmune serum reactive antigens or fragments thereof according to the present invention. This kind of nucleic acid molecule is particularly useful in the detection of a nucleic acid molecule according to the present invention and thus the diagnosis of the respective microorganisms such as *E. faecalis* and any disease or diseased condition where this kind of microorganisms is involved. Preferably, the hybridisation would occur or be preformed under stringent conditions as described in connection with the fourth alternative described above.

Nucleic acid molecule as used herein generally refers to any ribonucleic acid molecule or deoxyribonucleic acid molecule, which may be unmodified RNA or DNA or modified RNA or DNA. Thus, for instance, nucleic acid molecule as used herein refers to, among other, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded, or triple-stranded, or a mixture of single- and double-stranded regions. In addition, nucleic acid molecule as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. As used herein, the term nucleic acid molecule includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acid molecule" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are nucleic acid molecule as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term nucleic acid molecule as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of nucleic acid molecule, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, *inter alia*. The term nucleic acid molecule also embraces short nucleic acid molecules often referred to as oligonucleotide(s). "Polynucleotide" and "nucleic acid" or "nucleic acid molecule" are often used interchangeably herein.

Nucleic acid molecules provided in the present invention also encompass numerous unique fragments, both longer and shorter than the nucleic acid molecule sequences set forth in the sequencing listing of the

E. faecalis coding regions, which can be generated by standard cloning methods. To be unique, a fragment must be of sufficient size to distinguish it from other known nucleic acid sequences, most readily determined by comparing any selected *E. faecalis* fragment to the nucleotide sequences in computer databases such as GenBank.

Additionally, modifications can be made to the nucleic acid molecules and polypeptides that are encompassed by the present invention. For example, nucleotide substitutions can be made which do not affect the polypeptide encoded by the nucleic acid, and thus any nucleic acid molecule which encodes a hyperimmune serum reactive antigen or fragments thereof is encompassed by the present invention.

Furthermore, any of the nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof provided by the present invention can be functionally linked, using standard techniques such as standard cloning techniques, to any desired regulatory sequences, whether a *E. faecalis* regulatory sequence or a heterologous regulatory sequence, heterologous leader sequence, heterologous marker sequence or a heterologous coding sequence to create a fusion protein.

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA or cRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be triple-stranded, double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The present invention further relates to variants of the herein above described nucleic acid molecules which encode fragments, analogs and derivatives of the hyperimmune serum reactive antigens and fragments thereof having a deduced *E. faecalis* amino acid sequence set forth in the Sequence Listing. A variant of the nucleic acid molecule may be a naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Preferred are nucleic acid molecules encoding a variant, analog, derivative or fragment, or a variant, analogue or derivative of a fragment, which have a *E. faecalis* sequence as set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid(s) is substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the *E. faecalis* polypeptides set forth in the Sequence Listing. Also especially preferred in this regard are conservative substitutions.

The peptides and fragments according to the present invention also include modified epitopes wherein preferably one or two of the amino acids of a given epitope are modified or replaced according to the rules disclosed in e.g. [Tourdot, S. et al., 2000], as well as the nucleic acid sequences encoding such modified epitopes.

It is clear that also epitopes derived from the present epitopes by amino acid exchanges improving, conserving or at least not significantly impeding the T cell activating capability of the epitopes are covered by the epitopes according to the present invention. Therefore the present epitopes also cover epitopes, which do not contain the original sequence as derived from *E. faecalis*, but trigger the same or preferably an improved T cell response. These epitopes are referred to as "heteroclitic"; they need to have a

similar or preferably greater affinity to MHC/HLA molecules, and the need the ability to stimulate the T cell receptors (TCR) directed to the original epitope in a similar or preferably stronger manner.

Heteroclitic epitopes can be obtained by rational design i.e. taking into account the contribution of individual residues to binding to MHC/HLA as for instance described by {Rammensee, H. et al., 1999}, combined with a systematic exchange of residues potentially interacting with the TCR and testing the resulting sequences with T cells directed against the original epitope. Such a design is possible for a skilled man in the art without much experimentation.

Another possibility includes the screening of peptide libraries with T cells directed against the original epitope. A preferred way is the positional scanning of synthetic peptide libraries. Such approaches have been described in detail for instance by {Hemmer, B. et al., 1999} and the references given therein.

As an alternative to epitopes represented by the present derived amino acid sequences or heteroclitic epitopes, also substances mimicking these epitopes e.g. "peptidemimetica" or "retro-inverso-peptides" can be applied.

Another aspect of the design of improved epitopes is their formulation or modification with substances increasing their capacity to stimulate T cells. These include T helper cell epitopes, lipids or liposomes or preferred modifications as described in WO 01/78767.

Another way to increase the T cell stimulating capacity of epitopes is their formulation with immune stimulating substances for instance cytokines or chemokines like interleukin-2, -7, -12, -18, class I and II interferons (IFN), especially IFN-gamma, GM-CSF, TNF-alpha, flt3-ligand and others.

As discussed additionally herein regarding nucleic acid molecule assays of the invention, for instance, nucleic acid molecules of the invention as discussed above, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding polypeptides of the present invention and to isolate cDNA and genomic clones of other genes that have a high sequence similarity to the nucleic acid molecules of the present invention. Such probes generally will comprise at least 15 bases. Preferably, such probes will have at least 20, at least 25 or at least 30 bases, and may have at least 50 bases. Particularly preferred probes will have at least 30 bases, and will have 50 bases or less, such as 30, 35, 40, 45, or 50 bases.

For example, the coding region of a nucleic acid molecule of the present invention may be isolated by screening a relevant library using the known DNA sequence to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the present invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine to which members of the library the probe hybridizes.

The nucleic acid molecules and polypeptides of the present invention may be employed as reagents and materials for development of treatments of and diagnostics for disease, particularly human disease, as further discussed herein relating to nucleic acid molecule assays, *inter alia*.

The nucleic acid molecules of the present invention that are oligonucleotides can be used in the processes herein as described, but preferably for PCR, to determine whether or not the *E. faecalis* genes identified herein in whole or in part are present and/or transcribed in infected tissue such as blood. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained. For this and other purposes the arrays comprising at least one of the nucleic acids according to the present invention as described herein, may be used.

The nucleic acid molecules according to the present invention may be used for the detection of nucleic acid molecules and organisms or samples containing these nucleic acids. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease related or linked to the presence or abundance of *E. faecalis*.

Eukaryotes (herein also "individual(s)"), particularly mammals, and especially humans, infected with *E. faecalis* may be identifiable by detecting any of the nucleic acid molecules according to the present invention detected at the DNA level by a variety of techniques. Preferred nucleic acid molecules candidates for distinguishing a *E. faecalis* from other organisms can be obtained.

The invention provides a process for diagnosing disease, arising from infection with *E. faecalis*, comprising determining from a sample isolated or derived from an individual an increased level of expression of a nucleic acid molecule having the sequence of a nucleic acid molecule set forth in the Sequence Listing. Expression of nucleic acid molecules can be measured using any one of the methods well known in the art for the quantitation of nucleic acid molecules, such as, for example, PCR, RT-PCR, Rnase protection, Northern blotting, other hybridisation methods and the arrays described herein.

Isolated as used herein means separated "by the hand of man" from its natural state; i.e., that, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring nucleic acid molecule or a polypeptide naturally present in a living organism in its natural state is not "isolated," but the same nucleic acid molecule or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. As part of or following isolation, such nucleic acid molecules can be joined to other nucleic acid molecules, such as DNAs, for mutagenesis, to form fusion proteins, and for propagation or expression in a host, for instance. The isolated nucleic acid molecules, alone or joined to other nucleic acid molecules such as vectors, can be introduced into host cells, in culture or in whole organisms. Introduced into host cells in culture or in whole organisms, such DNAs still would be isolated, as the term is used herein, because they would not be in their naturally occurring form or environment. Similarly, the nucleic acid molecules and polypeptides may occur in a composition, such as a media formulations, solutions for introduction of nucleic acid molecules or polypeptides, for example, into cells, compositions or solutions for chemical or enzymatic reactions, for instance, which are not naturally occurring compositions, and, therein remain isolated nucleic acid molecules or polypeptides within the meaning of that term as it is employed herein.

The nucleic acids according to the present invention may be chemically synthesized. Alternatively, the nucleic acids can be isolated from *E. faecalis* by methods known to the one skilled in the art.

According to another aspect of the present invention, a comprehensive set of novel hyperimmune serum reactive antigens and fragments thereof are provided by using the herein described antigen identification method. In a preferred embodiment of the invention, a hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by any one of the nucleic acids molecules herein described and fragments thereof are provided. In another preferred embodiment of the invention a novel set of hyperimmune serum-reactive antigens which comprises amino acid sequences selected from a group consisting of the polypeptide sequences as represented in Seq ID No 171-172, 174-178, 180, 182-188, 190-193, 195-196, 199-213, 215-232, 234-244, 246-247, 249-253, 255-259, 261-262, 264-284, 287-296, 298-316, 318-340, 425, 427, 431-433, 439, 444, 446, 449-451, 459-460, 462-463 and 467-476 and fragments thereof are provided. In a further preferred embodiment of the invention hyperimmune serum-reactive antigens which comprise amino acid sequences selected from a group consisting of the polypeptide sequences as represented in Seq ID No 173, 179, 181, 194, 197, 214, 233, 245, 254, 285-286, 297, 426, 428-430, 434-438, 440-443, 445, 447-448, 452-458, 461 and 464-466 and fragments thereof are provided. In a still preferred embodiment of the invention hyperimmune serum-reactive antigens which comprise amino acid sequences selected from a group consisting of the polypeptide sequences as represented in Seq ID No 260, 317 and fragments thereof are provided.

The hyperimmune serum reactive antigens and fragments thereof as provided in the invention include any polypeptide set forth in the Sequence Listing as well as polypeptides which have at least 70% identity to a polypeptide set forth in the Sequence Listing, preferably at least 80% or 85% identity to a polypeptide set forth in the Sequence Listing, and more preferably at least 90% similarity (more preferably at least 90% identity) to a polypeptide set forth in the Sequence Listing and still more preferably at least 95%, 96%, 97%, 98%, 99% or 99.5% similarity (still more preferably at least 95%, 96%, 97%, 98%, 99%, or 99.5% identity) to a polypeptide set forth in the Sequence Listing and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 4 amino acids and more preferably at least 8, still more preferably at least 30, still more preferably at least 50 amino acids, such as 4, 8, 10, 20, 30, 35, 40, 45 or 50 amino acids.

The invention also relates to fragments, analogs, and derivatives of these hyperimmune serum reactive antigens and fragments thereof. The terms "fragment", "derivative" and "analog" when referring to an antigen whose amino acid sequence is set forth in the Sequence Listing, means a polypeptide which retains essentially the same or a similar biological function or activity as such hyperimmune serum reactive antigen and fragment thereof.

The fragment, derivative or analog of a hyperimmune serum reactive antigen and fragment thereof may be 1) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or 2) one in which one or more of the amino acid residues includes a substituent group, or 3) one in which the mature hyperimmune serum reactive antigen or fragment thereof is fused with another compound, such as a compound to increase the half-life of the hyperimmune serum reactive antigen and fragment thereof (for example, polyethylene glycol), or 4) one in which the additional amino acids are fused to the mature hyperimmune serum reactive antigen or fragment thereof, such as a leader or secretory sequence or a sequence which is employed for purification of the mature hyperimmune serum reactive antigen or fragment thereof or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The present invention also relates to antigens of different *E. faecalis* isolates. Such homologues may easily be isolated based on the nucleic acid and amino acid sequences disclosed herein. The presence of any antigen can accordingly be determined for every M serotype. In addition it is possible to determine the variability of a particular antigen in the various serotypes as described for the sic gene [Hoe, N. et al., 2001]. The contribution of the various serotypes to the different enterococcal infections varies in the different age groups and geographical regions. It is an important aspect that the most valuable protective antigens are expected to be conserved among various clinical strains.

Among the particularly preferred embodiments of the invention in this regard are the hyperimmune serum reactive antigens set forth in the Sequence Listing, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of fragments. Additionally, fusion polypeptides comprising such hyperimmune serum reactive antigens, variants, analogs, derivatives and fragments thereof, and variants, analogs and derivatives of the fragments are also encompassed by the present invention. Such fusion polypeptides and proteins, as well as nucleic acid molecules encoding them, can readily be made using standard techniques, including standard recombinant techniques for producing and expression of a recombinant polynucleic acid encoding a fusion protein.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another,

among the aliphatic amino acids Ala, Val, Leu and Ile; interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe and Tyr.

Further particularly preferred in this regard are variants, analogs, derivatives and fragments, and variants, analogs and derivatives of the fragments, having the amino acid sequence of any polypeptide set forth in the Sequence Listing, in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, deleted or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the polypeptide of the present invention. Also especially preferred in this regard are conservative substitutions. Most highly preferred are polypeptides having an amino acid sequence set forth in the Sequence Listing without substitutions.

The hyperimmune serum reactive antigens and fragments thereof of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

Also among preferred embodiments of the present invention are polypeptides comprising fragments of the polypeptides having the amino acid sequence set forth in the Sequence Listing, and fragments of variants and derivatives of the polypeptides set forth in the Sequence Listing.

In this regard a fragment is a polypeptide having an amino acid sequence that entirely is the same as part but not all of the amino acid sequence of the afore mentioned hyperimmune serum reactive antigen and fragment thereof, and variants or derivative, analogs, fragments thereof. Such fragments may be "free-standing", i.e., not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. Also preferred in this aspect of the invention are fragments characterised by structural or functional attributes of the polypeptide of the present invention, i.e. fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta-amphipathic regions, flexible regions, surface-forming regions, substrate binding regions, and high antigenic index regions of the polypeptide of the present invention, and combinations of such fragments. Preferred regions are those that mediate activities of the hyperimmune serum reactive antigens and fragments thereof of the present invention. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of the hyperimmune serum reactive antigen and fragments thereof of the present invention, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Particularly preferred are fragments comprising receptors or domains of enzymes that confer a function essential for viability of *E. faecalis* or the ability to cause disease in humans. Further preferred polypeptide fragments are those that comprise or contain antigenic or immunogenic determinants in an animal, especially in a human.

An antigenic fragment is defined as a fragment of the identified antigen which is for itself antigenic or may be made antigenic when provided as a hapten. Therefore, also antigens or antigenic fragments showing one or (for longer fragments) only a few amino acid exchanges are enabled with the present invention, provided that the antigenic capacities of such fragments with amino acid exchanges are not severely deteriorated on the exchange(s), i.e., suited for eliciting an appropriate immune response in an individual vaccinated with this antigen and identified by individual antibody preparations from individual sera.

Preferred examples of such fragments of a hyperimmune serum-reactive antigen are selected from the group consisting of peptides comprising amino acid sequences of column "predicted immunogenic aa", and "Location of identified immunogenic region" of Table 1a and Table 1c; the serum reactive epitopes of Table 2, especially peptides comprising amino acid 4-10, 14-21, 30-36, 59-68, 77-82, 87-93, 96-105, 112-121,

125-133, 135-141, 150-162, 164-183, 192-203, 207-213, 215-226, 228-234, 241-247, 250-285, 302-308 and 135-148 of Seq ID No 171; 15-57, 60-73, 77-101, 108-134, 136-177, 185-201, 203-217, 226-240, 244-254, 272-277, 283-288, 292-343, 354-370, 380-398, 406-437, 439-453, 473-490, 532-538, 584-590, 595-601, 606-612, 664-677, 679-704, 715-724, 731-753, 759-772, 786-794, 814-862 and 657-684 of Seq ID No 172; 4-9, 15-36, 41-47, 54-60, 75-81, 114-120, 131-146, 152-158, 174-182, 194-202, 208-215, 218-226, 255-271, 276-285, 290-295, 302-311, 318-328, 330-344, 352-359, 365-377, 388-395, 398-405, 426-432, 439-449, 455-500, 505-513, 531-537, 542-552, 554-561, 587-595, 606-612, 718-734, 763-771, 775-782, 792-801, 805-812, 822-828, 830-843, 849-863, 876-894, 905-911, 919-926, 935-947, 949-958, 968-979, 1009-1016, 1029-1045, 1047-1056, 1076-1081, 1092-1106, 1123-1133, 1179-1200, 1202-1211, 1215-1223, 1287-1299, 1301-1306, 398-431 and 1224-1237 of Seq ID No 173; 17-47, 74-80, 90-97, 126-133, 137-148, 167-173, 179-185, 214-223, 250-255, 270-283, 329-338, 342-350, 352-358, 360-367, 372-383, 398-404, 411-421, 426-432, 435-446, 452-462, 472-479, 515-521, 582-592, 611-618, 623-629, 642-659, 666-673, 678-689, 704-725, 732-737, 744-757, 768-789, 824-834, 842-849, 862-868, 877-887, 904-916, 923-928, 941-947, 962-974, 982-992, 1019-1030, 1032-1044, 1046-1052, 1065-1075, 1077-1087, 1108-1121, 1124-1132, 1137-1151, 1170-1182, 1190-1206, 1208-1214, 1227-1233, 1242-1251, 1254-1273, 1282-1298 and 792-825 of Seq ID No 174; 19-31, 39-67, 82-91, 104-110, 113-128, 149-155, 161-181 and 137-155 of Seq ID No 175; 6-18, 54-63, 69-85, 110-127, 142-156, 158-167, 169-211, 238-246, 248-257, 276-311, 339-349, 371-380, 385-391, 394-403, 421-438, 451-456, 483-489 and 449-468 of Seq ID No 176; 5-15, 24-34, 50-56, 61-83, 98-121, 123-136, 149-162, 166-194, 202-215, 221-227, 229-332, 337-360, 367-402, 404-415, 427-433, 444-462, 471-478, 487-498, 511-518, 521-544, 550-563, 568-574, 580-587, 597-607, 610-616, 624-629 and 468-498 of Seq ID No 177; 11-19, 32-49, 57-63, 65-71, 80-89, 91-133, 166-181, 183-191, 201-230, 234-257, 264-291, 297-303, 305-314, 316-335, 337-354, 359-366, 368-374, 383-388, 394-405, 408-442, 446-470, 483-490, 499-505, 513-538, 544-555, 557-563, 568-590, 598-608, 617-623, 627-636, 641-647, 667-685, 687-693, 710-723, 733-739, 742-754, 769-815 and 366-388 of Seq ID No 178; 4-16, 30-35, 42-53, 67-76, 82-87, 101-108, 112-130, 132-138, 147-152, 161-183, 187-208, 218-225, 265-281, 295-303, 305-317, 322-334, 338-357, 360-368, 370-383, 387-394, 400-419, 421-430 and 255-336 of Seq ID No 179; 19-27, 36-47, 59-66, 76-83, 101-112, 118-125, 142-147, 162-180, 185-196, 225-240, 246-263, 286-304, 314-319, 327-333, 353-367 and 194-214 of Seq ID No 180; 14-43, 70-76, 83-89, 111-117, 122-128, 136-145, 163-170, 175-182, 210-219, 246-251, 266-279, 325-331, 338-346, 348-354, 356-363, 368-379, 422-428, 431-441, 450-456, 466-473, 509-515, 532-542, 549-556, 576-586, 605-612, 617-623, 636-653, 660-667, 674-686, 698-719, 726-731, 738-745, 762-783, 818-828, 836-843, 856-862, 871-881, 903-910, 917-922, 935-941, 956-968, 976-986, 1013-1024, 1026-1038, 1059-1069, 1071-1081, 1102-1115, 1118-1126, 1131-1145, 1164-1176, 1187-1200, 1202-1208, 1221-1227, 1236-1245, 1248-1267, 1273-1292, 252-287 and 805-844 of Seq ID No 181; 4-18, 21-28, 37-43, 56-70, 101-113, 131-140, 142-150, 162-170, 172-184, 193-204, 209-227, 233-238, 246-264 and 93-168 of Seq ID No 182; 14-20, 44-50, 61-70, 77-96, 99-106, 129-142, 168-181, 187-196, 205-221, 225-241, 277-296 and 257-281 of Seq ID No 183; 18-29, 43-54, 64-76, 78-84, 88-103, 125-149, 159-176, 198-218, 230-242, 256-271, 279-285, 287-293, 300-306, 325-331, 344-351, 357-364, 371-397, 400-414, 419-464, 485-515, 517-526, 529-537, 548-553, 573-580, 584-590, 603-620, 639-661, 676-681, 687-700, 716-761, 772-780, 785-790, 795-803, 823-836, 848-853 and 106-134 of Seq ID No 184; 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All linear hyperimmune serum reactive fragments of a particular antigen may be identified by analysing the entire sequence of the protein antigen by a set of peptides overlapping by 1 amino acid with a length of at least 10 amino acids. Subsequently, non-linear epitopes can be identified by analysis of the protein antigen with hyperimmune sera using the expressed full-length protein or domain polypeptides thereof. Assuming that a distinct domain of a protein is sufficient to form the 3D structure independent from the native protein, the analysis of the respective recombinant or synthetically produced domain polypeptide with hyperimmune serum would allow the identification of conformational epitopes within the individual domains of multi-domain proteins. For those antigens where a domain possesses linear as well as conformational epitopes, competition experiments with peptides corresponding to the linear epitopes may be used to confirm the presence of conformational epitopes.

It will be appreciated that the invention also relates to, among others, nucleic acid molecules encoding the aforementioned fragments, nucleic acid molecules that hybridise to nucleic acid molecules encoding the fragments, particularly those that hybridise under stringent conditions, and nucleic acid molecules, such as PCR primers, for amplifying nucleic acid molecules that encode the fragments. In these regards, preferred nucleic acid molecules are those that correspond to the preferred fragments, as discussed above.

The present invention also relates to vectors which comprise a nucleic acid molecule or nucleic acid molecules of the present invention, host cells which are genetically engineered with vectors of the invention and the production of hyperimmune serum reactive antigens and fragments thereof by recombinant techniques.

A great variety of expression vectors can be used to express a hyperimmune serum reactive antigen or fragment thereof according to the present invention. Generally, any vector suitable to maintain, propagate or express nucleic acids to express a polypeptide in a host may be used for expression in this regard. In accordance with this aspect of the invention the vector may be, for example, a plasmid vector, a single or double-stranded phage vector, a single or double-stranded RNA or DNA viral vector. Starting plasmids disclosed herein are either commercially available, publicly available, or can be constructed from available plasmids by routine application of well-known, published procedures. Preferred among vectors, in certain respects, are those for expression of nucleic acid molecules and hyperimmune serum

reactive antigens or fragments thereof of the present invention. Nucleic acid constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the hyperimmune serum reactive antigens and fragments thereof of the invention can be synthetically produced by conventional peptide synthesizers. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA construct of the present invention.

Host cells can be genetically engineered to incorporate nucleic acid molecules and express nucleic acid molecules of the present invention. Representative examples of appropriate hosts include bacterial cells, such as enterococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, 293 and Bowes melanoma cells; and plant cells.

The invention also provides a process for producing a *E. faecalis* hyperimmune serum reactive antigen and a fragment thereof comprising expressing from the host cell a hyperimmune serum reactive antigen or fragment thereof encoded by the nucleic acid molecules provided by the present invention. The invention further provides a process for producing a cell, which expresses a *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof comprising transforming or transfecting a suitable host cell with the vector according to the present invention such that the transformed or transfected cell expresses the polypeptide encoded by the nucleic acid contained in the vector.

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N- or C-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, regions may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability or to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize or purify polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another protein or part thereof. In drug discovery, for example, proteins have been fused with antibody Fc portions for the purpose of high-throughout screening assays to identify antagonists. See for example, {Bennett, D. et al., 1995} and {Johanson, K. et al., 1995}.

The *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention can be produced by chemical synthesis as well as by biotechnological means. The latter comprise the transfection or transformation of a host cell with a vector containing a nucleic acid according to the present invention and the cultivation of the transfected or transformed host cell under conditions which are known to the ones skilled in the art. The production method may also comprise a purification step in order to purify or isolate the polypeptide to be manufactured. In a preferred embodiment the vector is a vector according to the present invention.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention may be used for the detection of the organism or organisms in a sample containing these organisms or polypeptides derived thereof. Preferably such detection is for diagnosis, more preferable for the diagnosis of a disease, most preferably for the diagnosis of a diseases related or linked to the presence or abundance of Gram-positive bacteria, especially bacteria selected from the group comprising enterococci, staphylococci and lactococci. More preferably, the microorganisms are selected from the group comprising *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Streptococcus mutans*, especially the microorganism is *Enterococcus faecalis*.

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of the hyperimmune serum reactive antigens and fragments thereof of the present invention in cells and tissues, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of the polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example, and to identify the infecting organism. Assay techniques that can be used to determine levels of a polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Among these, ELISAs frequently are preferred. An ELISA assay initially comprises preparing an antibody specific to the polypeptide, preferably a monoclonal antibody. In addition, a reporter antibody generally is prepared which binds to the monoclonal antibody. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, such as horseradish peroxidase enzyme.

The hyperimmune serum reactive antigens and fragments thereof according to the present invention may also be used for the purpose of or in connection with an array. More particularly, at least one of the hyperimmune serum reactive antigens and fragments thereof according to the present invention may be immobilized on a support. Said support typically comprises a variety of hyperimmune serum reactive antigens and fragments thereof whereby the variety may be created by using one or several of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and/or hyperimmune serum reactive antigens and fragments thereof being different. The characterizing feature of such array as well as of any array in general is the fact that at a distinct or predefined region or position on said support or a surface thereof, a distinct polypeptide is immobilized. Because of this any activity at a distinct position or region of an array can be correlated with a specific polypeptide. The number of different hyperimmune serum reactive antigens and fragments thereof immobilized on a support may range from as little as 10 to several 1000 different hyperimmune serum reactive antigens and fragments thereof. The density of hyperimmune serum reactive antigens and fragments thereof per cm^2 is in a preferred embodiment as little as 10 peptides/polypeptides per cm^2 to at least 400 different peptides/polypeptides per cm^2 and more particularly at least 1000 different hyperimmune serum reactive antigens and fragments thereof per cm^2 .

The manufacture of such arrays is known to the one skilled in the art and, for example, described in US patent 5,744,309. The array preferably comprises a planar, porous or non-porous solid support having at least a first surface. The hyperimmune serum reactive antigens and fragments thereof as disclosed herein, are immobilized on said surface. Preferred support materials are, among others, glass or cellulose. It is also within the present invention that the array is used for any of the diagnostic applications described herein. Apart from the hyperimmune serum reactive antigens and fragments thereof according to the present invention also the nucleic acid molecules according to the present invention may be used for the generation of an array as described above. This applies as well to an array made of antibodies, preferably monoclonal antibodies as, among others, described herein.

In a further aspect the present invention relates to an antibody directed to any of the hyperimmune serum reactive antigens and fragments thereof, derivatives or fragments thereof according to the present

invention. The present invention includes, for example, monoclonal and polyclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of a Fab expression library. It is within the present invention that the antibody may be chimeric, i. e. that different parts thereof stem from different species or at least the respective sequences are taken from different species.

Antibodies generated against the hyperimmune serum reactive antigens and fragments thereof corresponding to a sequence of the present invention can be obtained by direct injection of the hyperimmune serum reactive antigens and fragments thereof into an animal or by administering the hyperimmune serum reactive antigens and fragments thereof to an animal, preferably a non-human. The antibody so obtained will then bind the hyperimmune serum reactive antigens and fragments thereof itself. In this manner, even a sequence encoding only a fragment of a hyperimmune serum reactive antigen and fragments thereof can be used to generate antibodies binding the whole native hyperimmune serum reactive antigen and fragments thereof. Such antibodies can then be used to isolate the hyperimmune serum reactive antigens and fragments thereof from tissue expressing those hyperimmune serum reactive antigens and fragments thereof.

For preparation of monoclonal antibodies, any technique known in the art which provides antibodies produced by continuous cell line cultures can be used (as described originally in [Kohler, G. et al., 1975]).

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to immunogenic hyperimmune serum reactive antigens and fragments thereof according to this invention. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies to immunogenic hyperimmune serum reactive antigens and fragments thereof according to this invention.

Alternatively, phage display technology or ribosomal display could be utilized to select antibody genes with binding activities towards the hyperimmune serum reactive antigens and fragments thereof either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing respective target antigens or from naïve libraries [McCafferty, J. et al., 1990]; [Marks, J. et al., 1992]. The affinity of these antibodies can also be improved by chain shuffling [Clackson, T. et al., 1991].

If two antigen binding domains are present, each domain may be directed against a different epitope – termed ‘bispecific’ antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the hyperimmune serum reactive antigens and fragments thereof or purify the hyperimmune serum reactive antigens and fragments thereof of the present invention by attachment of the antibody to a solid support for isolation and/or purification by affinity chromatography.

Thus, among others, antibodies against the hyperimmune serum reactive antigens and fragments thereof of the present invention may be employed to inhibit and/or treat infections, particularly bacterial infections and especially infections arising from *E. faecalis*.

Hyperimmune serum reactive antigens and fragments thereof include antigenically, epitopically or immunologically equivalent derivatives which form a particular aspect of this invention. The term “antigenically equivalent derivative” as used herein encompasses a hyperimmune serum reactive antigen and fragments thereof or its equivalent which will be specifically recognized by certain antibodies which, when raised to the protein or hyperimmune serum reactive antigen and fragments thereof according to the present invention, interfere with the interaction between pathogen and mammalian host. The term “immunologically equivalent derivative” as used herein encompasses a peptide or its equivalent which when used in a suitable formulation to raise antibodies in a vertebrate, the antibodies act to interfere with the interaction between pathogen and mammalian host.

The hyperimmune serum reactive antigens and fragments thereof, such as an antigenically or immunologically equivalent derivative or a fusion protein thereof can be used as an antigen to immunize a mouse or other animal such as a rat or chicken. The fusion protein may provide stability to the hyperimmune serum reactive antigens and fragments thereof. The antigen may be associated, for example by conjugation, with an immunogenic carrier protein, for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively, an antigenic peptide comprising multiple copies of the protein or hyperimmune serum reactive antigen and fragments thereof, or an antigenically or immunologically equivalent hyperimmune serum reactive antigen and fragments thereof, may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

Preferably the antibody or derivative thereof is modified to make it less immunogenic in the individual. For example, if the individual is human the antibody may most preferably be "humanized", wherein the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in [Jones, P. et al., 1986] or [Tempest, P. et al., 1991].

The use of a polynucleotide of the invention in genetic immunization will preferably employ a suitable delivery method such as direct injection of plasmid DNA into muscle, delivery of DNA complexed with specific protein carriers, coprecipitation of DNA with calcium phosphate, encapsulation of DNA in various forms of liposomes, particle bombardment [Tang, D. et al., 1992]; [Eisenbraun, M. et al., 1993] and *in vivo* infection using cloned retroviral vectors [Seeger, C. et al., 1984].

In a further aspect the present invention relates to a peptide binding to any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, and a method for the manufacture of such peptides whereby the method is characterized by the use of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and the basic steps are known to the one skilled in the art.

Such peptides may be generated by using methods according to the state of the art such as phage display or ribosome display. In case of phage display, basically a library of peptides is generated, in form of phages, and this kind of library is contacted with the target molecule, in the present case a hyperimmune serum reactive antigen and fragments thereof according to the present invention. Those peptides binding to the target molecule are subsequently removed, preferably as a complex with the target molecule, from the respective reaction. It is known to the one skilled in the art that the binding characteristics, at least to a certain extent, depend on the particularly realized experimental set-up such as the salt concentration and the like. After separating those peptides binding to the target molecule with a higher affinity or a bigger force, from the non-binding members of the library, and optionally also after removal of the target molecule from the complex of target molecule and peptide, the respective peptide(s) may subsequently be characterised. Prior to the characterisation optionally an amplification step is realized such as, e. g. by propagating the peptide encoding phages. The characterisation preferably comprises the sequencing of the target binding peptides. Basically, the peptides are not limited in their lengths, however, preferably peptides having a lengths from about 8 to 20 amino acids are preferably obtained in the respective methods. The size of the libraries may be about 10^2 to 10^{18} , preferably 10^8 to 10^{15} different peptides, however, is not limited thereto.

A particular form of target binding hyperimmune serum reactive antigens and fragments thereof are the so-called "anticalines" which are, among others, described in German patent application DE 197 42 706.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, and a method for the manufacture of such functional nucleic acids whereby the method is characterized by the

use of the hyperimmune serum reactive antigens and fragments thereof according to the present invention and the basic steps are known to the one skilled in the art. The functional nucleic acids are preferably aptamers and spiegelmers.

Aptamers are D-nucleic acids which are either single stranded or double stranded and which specifically interact with a target molecule. The manufacture or selection of aptamers is, e. g., described in European patent EP 0 533 838. Basically the following steps are realized. First, a mixture of nucleic acids, i. e. potential aptamers, is provided whereby each nucleic acid typically comprises a segment of several, preferably at least eight subsequent randomised nucleotides. This mixture is subsequently contacted with the target molecule whereby the nucleic acid(s) bind to the target molecule, such as based on an increased affinity towards the target or with a bigger force thereto, compared to the candidate mixture. The binding nucleic acid(s) are/is subsequently separated from the remainder of the mixture. Optionally, the thus obtained nucleic acid(s) is amplified using, e.g. polymerase chain reaction. These steps may be repeated several times giving at the end a mixture having an increased ratio of nucleic acids specifically binding to the target from which the final binding nucleic acid is then optionally selected. These specifically binding nucleic acid(s) are referred to as aptamers. It is obvious that at any stage of the method for the generation or identification of the aptamers samples of the mixture of individual nucleic acids may be taken to determine the sequence thereof using standard techniques. It is within the present invention that the aptamers may be stabilized such as, e. g., by introducing defined chemical groups which are known to the one skilled in the art of generating aptamers. Such modification may for example reside in the introduction of an amino group at the 2'-position of the sugar moiety of the nucleotides. Aptamers are currently used as therapeutical agents. However, it is also within the present invention that the thus selected or generated aptamers may be used for target validation and/or as lead substance for the development of medicaments, preferably of medicaments based on small molecules. This is actually done by a competition assay whereby the specific interaction between the target molecule and the aptamer is inhibited by a candidate drug whereby upon replacement of the aptamer from the complex of target and aptamer it may be assumed that the respective drug candidate allows a specific inhibition of the interaction between target and aptamer, and if the interaction is specific, said candidate drug will, at least in principle, be suitable to block the target and thus decrease its biological availability or activity in a respective system comprising such target. The thus obtained small molecule may then be subject to further derivatisation and modification to optimise its physical, chemical, biological and/or medical characteristics such as toxicity, specificity, biodegradability and bioavailability.

Spiegelmers and their generation or manufacture is based on a similar principle. The manufacture of spiegelmers is described in international patent application WO 98/08856. Spiegelmers are L-nucleic acids, which means that they are composed of L-nucleotides rather than D-nucleotides as aptamers are. Spiegelmers are characterized by the fact that they have a very high stability in biological systems and, comparable to aptamers, specifically interact with the target molecule against which they are directed. In the process of generating spiegelmers, a heterogeneous population of D-nucleic acids is created and this population is contacted with the optical antipode of the target molecule, in the present case for example with the D-enantiomer of the naturally occurring L-enantiomer of the hyperimmune serum reactive antigens and fragments thereof according to the present invention. Subsequently, those D-nucleic acids are separated which do not interact with the optical antipode of the target molecule. But those D-nucleic acids interacting with the optical antipode of the target molecule are separated, optionally identified and/or sequenced and subsequently the corresponding L-nucleic acids are synthesized based on the nucleic acid sequence information obtained from the D-nucleic acids. These L-nucleic acids which are identical in terms of sequence with the aforementioned D-nucleic acids interacting with the optical antipode of the target molecule, will specifically interact with the naturally occurring target molecule rather than with the optical antipode thereof. Similar to the method for the generation of aptamers it is also possible to repeat the various steps several times and thus to enrich those nucleic acids specifically interacting with the optical antipode of the target molecule.

In a further aspect the present invention relates to functional nucleic acids interacting with any of the nucleic acid molecules according to the present invention, and a method for the manufacture of such functional nucleic acids whereby the method is characterized by the use of the nucleic acid molecules and their respective sequences according to the present invention and the basic steps are known to the one skilled in the art. The functional nucleic acids are preferably ribozymes, antisense oligonucleotides and siRNA.

Ribozymes are catalytically active nucleic acids which preferably consist of RNA which basically comprises two moieties. The first moiety shows a catalytic activity whereas the second moiety is responsible for the specific interaction with the target nucleic acid, in the present case the nucleic acid coding for the hyperimmune serum reactive antigens and fragments thereof according to the present invention. Upon interaction between the target nucleic acid and the second moiety of the ribozyme, typically by hybridisation and Watson-Crick base pairing of essentially complementary stretches of bases on the two hybridising strands, the catalytically active moiety may become active which means that it catalyses, either intramolecularly or intermolecularly, the target nucleic acid in case the catalytic activity of the ribozyme is a phosphodiesterase activity. Subsequently, there may be a further degradation of the target nucleic acid which in the end results in the degradation of the target nucleic acid as well as the protein derived from the said target nucleic acid. Ribozymes, their use and design principles are known to the one skilled in the art, and, for example described in {Doherty, E. et al., 2001} and {Lewin, A. et al., 2001}.

The activity and design of antisense oligonucleotides for the manufacture of a medicament and as a diagnostic agent, respectively, is based on a similar mode of action. Basically, antisense oligonucleotides hybridise based on base complementarity, with a target RNA, preferably with a mRNA, thereby activating RNase H. RNase H is activated by both phosphodiester and phosphorothioate-coupled DNA. Phosphodiester-coupled DNA, however, is rapidly degraded by cellular nucleases with the exception of phosphorothioate-coupled DNA. These resistant, non-naturally occurring DNA derivatives do not inhibit RNase H upon hybridisation with RNA. In other words, antisense polynucleotides are only effective as DNA RNA hybrid complexes. Examples for this kind of antisense oligonucleotides are described, among others, in US-patent US 5,849,902 and US 5,989,912. In other words, based on the nucleic acid sequence of the target molecule which in the present case are the nucleic acid molecules for the hyperimmune serum reactive antigens and fragments thereof according to the present invention, either from the target protein from which a respective nucleic acid sequence may in principle be deduced, or by knowing the nucleic acid sequence as such, particularly the mRNA, suitable antisense oligonucleotides may be designed base on the principle of base complementarity.

Particularly preferred are antisense-oligonucleotides which have a short stretch of phosphorothioate DNA (3 to 9 bases). A minimum of 3 DNA bases is required for activation of bacterial RNase H and a minimum of 5 bases is required for mammalian RNase H activation. In these chimeric oligonucleotides there is a central region that forms a substrate for RNase H that is flanked by hybridising "arms" comprised of modified nucleotides that do not form substrates for RNase H. The hybridising arms of the chimeric oligonucleotides may be modified such as by 2'-O-methyl or 2'-fluoro. Alternative approaches used methylphosphonate or phosphoramidate linkages in said arms. Further embodiments of the antisense oligonucleotide useful in the practice of the present invention are P-methoxyoligonucleotides, partial P-methoxyoligodeoxyribonucleotides or P-methoxyoligonucleotides.

Of particular relevance and usefulness for the present invention are those antisense oligonucleotides as more particularly described in the above two mentioned US patents. These oligonucleotides contain no naturally occurring 5'→3'-linked nucleotides. Rather the oligonucleotides have two types of nucleotides: 2'-deoxyphosphorothioate, which activate RNase H, and 2'-modified nucleotides, which do not. The linkages between the 2'-modified nucleotides can be phosphodiester, phosphorothioate or P-ethoxyphosphodiester. Activation of RNase H is accomplished by a contiguous RNase H-activating

region, which contains between 3 and 5 2'-deoxyphosphorothioate nucleotides to activate bacterial RNase H and between 5 and 10 2'- deoxyphosphorothioate nucleotides to activate eucaryotic and, particularly, mammalian RNase H. Protection from degradation is accomplished by making the 5' and 3' terminal bases highly nuclease resistant and, optionally, by placing a 3' terminal blocking group.

More particularly, the antisense oligonucleotide comprises a 5' terminus and a 3' terminus; and from position 11 to 59 5'→3'-linked nucleotides independently selected from the group consisting of 2'-modified phosphodiester nucleotides and 2'-modified P-alkyloxyphosphotriester nucleotides; and wherein the 5'-terminal nucleoside is attached to an RNase H-activating region of between three and ten contiguous phosphorothioate-linked deoxyribonucleotides, and wherein the 3'-terminus of said oligonucleotide is selected from the group consisting of an inverted deoxyribonucleotide, a contiguous stretch of one to three phosphorothioate 2'-modified ribonucleotides, a biotin group and a P-alkyloxyphosphotriester nucleotide.

Also an antisense oligonucleotide may be used wherein not the 5' terminal nucleoside is attached to an RNase H-activating region but the 3' terminal nucleoside as specified above. Also, the 5' terminus is selected from the particular group rather than the 3' terminus of said oligonucleotide.

The nucleic acids as well as the hyperimmune serum reactive antigens and fragments thereof according to the present invention may be used as or for the manufacture of pharmaceutical compositions, especially vaccines. Preferably such pharmaceutical composition, preferably vaccine is for the prevention or treatment of diseases caused by, related to or associated with *E. faecalis*. In so far another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, which comprises inoculating the individual with the hyperimmune serum reactive antigens and fragments thereof of the invention, or a fragment or variant thereof, adequate to produce antibodies to protect said individual from infection, particularly enterococcal infection and most particularly *E. faecalis* infections.

Yet another aspect of the invention relates to a method of inducing an immunological response in an individual which comprises, through gene therapy or otherwise, delivering a nucleic acid functionally encoding hyperimmune serum reactive antigens and fragments thereof, or a fragment or a variant thereof, for expressing the hyperimmune serum reactive antigens and fragments thereof, or a fragment or a variant thereof *in vivo* in order to induce an immunological response to produce antibodies or a cell mediated T cell response, either cytokine-producing T cells or cytotoxic T cells, to protect said individual from disease, whether that disease is already established within the individual or not. One way of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise.

A further aspect of the invention relates to an immunological composition which, when introduced into a host capable of having induced within it an immunological response, induces an immunological response in such host, wherein the composition comprises recombinant DNA which codes for and expresses an antigen of the hyperimmune serum reactive antigens and fragments thereof of the present invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity or cellular immunity such as that arising from CTL or CD4+ T cells.

The hyperimmune serum reactive antigens and fragments thereof of the invention or a fragment thereof may be fused with a co-protein which may not by itself produce antibodies, but is capable of stabilizing the first protein and producing a fused protein which will have immunogenic and protective properties. This fused recombinant protein preferably further comprises an antigenic co-protein, such as Glutathione-S-transferase (GST) or beta-galactosidase, relatively large co-proteins which solubilise the protein and facilitate production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system. The co-protein may be attached to either the amino or carboxy terminus of the first protein.

Also, provided by this invention are methods using the described nucleic acid molecule or particular fragments thereof in such genetic immunization experiments in animal models of infection with *E. faecalis*. Such fragments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. This approach can allow for the subsequent preparation of monoclonal antibodies of particular value from the requisite organ of the animal successfully resisting or clearing infection for the development of prophylactic agents or therapeutic treatments of enterococcal infection in mammals, particularly humans.

The hyperimmune serum reactive antigens and fragments thereof may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue. Examples of tissue damage include wounds in skin or connective tissue caused e.g. by mechanical, chemical or thermal damage or by implantation of indwelling devices, or wounds in the mucous membranes, such as the mouth, mammary glands, urethra or vagina.

The present invention also includes a vaccine formulation which comprises the immunogenic recombinant protein together with a suitable carrier. Since the protein may be broken down in the stomach, it is preferably administered parenterally, including, for example, administration that is subcutaneous, intramuscular, intravenous, intradermal intranasal or transdermal. Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the bodily fluid, preferably the blood, of the individual; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials, and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in-water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

According to another aspect, the present invention relates to a pharmaceutical composition comprising such a hyperimmune serum-reactive antigen or a fragment thereof as provided in the present invention for *E. faecalis*. Such a pharmaceutical composition may comprise one, preferably at least two, or more hyperimmune serum reactive antigens or fragments thereof against *E. faecalis*. Optionally, such *E. faecalis* hyperimmune serum reactive antigens or fragments thereof may also be combined with antigens against other pathogens in a combination pharmaceutical composition. Preferably, said pharmaceutical composition is a vaccine for preventing or treating an infection caused by *E. faecalis* and/or other pathogens against which the antigens have been included in the vaccine.

According to a further aspect, the present invention relates to a pharmaceutical composition comprising a nucleic acid molecule encoding a hyperimmune serum-reactive antigen or a fragment thereof as identified above for *E. faecalis*. Such a pharmaceutical composition may comprise one or more nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof against *E. faecalis*. Optionally, such *E. faecalis* nucleic acid molecules encoding hyperimmune serum reactive antigens or fragments thereof may also be combined with nucleic acid molecules encoding antigens against other pathogens in a combination pharmaceutical composition. Preferably, said pharmaceutical composition is a vaccine for preventing or treating an infection caused by *E. faecalis* and/or other pathogens against which the antigens have been included in the vaccine.

The pharmaceutical composition may contain any suitable auxiliary substances, such as buffer substances, stabilisers or further active ingredients, especially ingredients known in connection of pharmaceutical composition and/or vaccine production.

A preferable carrier/or excipient for the hyperimmune serum-reactive antigens, fragments thereof or a coding nucleic acid molecule thereof according to the present invention is an immunostimulatory compound for further stimulating the immune response to the given hyperimmune serum-reactive antigen, fragment thereof or a coding nucleic acid molecule thereof. Preferably the immunostimulatory compound in the pharmaceutical preparation according to the present invention is selected from the group of polycationic substances, especially polycationic peptides, immunostimulatory nucleic acids molecules, preferably immunostimulatory deoxynucleotides, alum, Freund's complete adjuvants, Freund's incomplete adjuvants, neuroactive compounds, especially human growth hormone, or combinations thereof.

It is also within the scope of the present invention that the pharmaceutical composition, especially vaccine, comprises apart from the hyperimmune serum reactive antigens, fragments thereof and/or coding nucleic acid molecules thereof according to the present invention other compounds which are biologically or pharmaceutically active. Preferably, the vaccine composition comprises at least one polycationic peptide. The polycationic compound(s) to be used according to the present invention may be any polycationic compound which shows the characteristic effects according to the WO 97/30721. Preferred polycationic compounds are selected from basic polypeptides, organic polycations, basic polyamino acids or mixtures thereof. These polyamino acids should have a chain length of at least 4 amino acid residues (WO 97/30721). Especially preferred are substances like polylysine, polyarginine and polypeptides containing more than 20 %, especially more than 50 % of basic amino acids in a range of more than 8, especially more than 20, amino acid residues or mixtures thereof. Other preferred polycations and their pharmaceutical compositions are described in WO 97/30721 (e.g. polyethyleneimine) and WO 99/38528. Preferably these polypeptides contain between 20 and 500 amino acid residues, especially between 30 and 200 residues.

These polycationic compounds may be produced chemically or recombinantly or may be derived from natural sources.

Cationic (poly)peptides may also be anti-microbial with properties as reviewed in (Ganz, T., 1999). These (poly)peptides may be of prokaryotic or animal or plant origin or may be produced chemically or recombinantly (WO 02/13857). Peptides may also belong to the class of defensins (WO 02/13857). Sequences of such peptides can be, for example, found in the Antimicrobial Sequences Database under the following internet address:

<http://www.bbcm.univ.trieste.it/~tossi/pag2.html>

Such host defence peptides or defensives are also a preferred form of the polycationic polymer according to the present invention. Generally, a compound allowing as an end product activation (or down-regulation) of the adaptive immune system, preferably mediated by APCs (including dendritic cells) is used as polycationic polymer.

Especially preferred for use as polycationic substances in the present invention are cathelicidin derived antimicrobial peptides or derivatives thereof (International patent application WO 02/13857, incorporated herein by reference), especially antimicrobial peptides derived from mammalian cathelicidin, preferably from human, bovine or mouse.

Polycationic compounds derived from natural sources include HIV-REV or HIV-TAT (derived cationic peptides, antennapedia peptides, chitosan or other derivatives of chitin) or other peptides derived from these peptides or proteins by biochemical or recombinant production. Other preferred polycationic compounds are cathelin or related or derived substances from cathelin. For example, mouse cathelin is a peptide which has the amino acid sequence NH₂-RLAGLLRKGGKIGEKLLKKIGOKIKNFFQKLVPQPE-

COOH. Related or derived cathelin substances contain the whole or parts of the cathelin sequence with at least 15-20 amino acid residues. Derivations may include the substitution or modification of the natural amino acids by amino acids which are not among the 20 standard amino acids. Moreover, further cationic residues may be introduced into such cathelin molecules. These cathelin molecules are preferred to be combined with the antigen. These cathelin molecules surprisingly have turned out to be also effective as an adjuvant for an antigen without the addition of further adjuvants. It is therefore possible to use such cathelin molecules as efficient adjuvants in vaccine formulations with or without further immunactivating substances.

Another preferred polycationic substance to be used according to the present invention is a synthetic peptide containing at least 2 KKK-motifs separated by a linker of 3 to 7 hydrophobic amino acids (International patent application WO 02/32451, incorporated herein by reference).

The pharmaceutical composition of the present invention may further comprise immunostimulatory nucleic acid(s). Immunostimulatory nucleic acids are e. g. neutral or artificial CpG containing nucleic acids, short stretches of nucleic acids derived from non-vertebrates or in form of short oligonucleotides (ODNs) containing non-methylated cytosine-guanine di-nucleotides (CpG) in a certain base context (e.g. described in WO 96/02555). Alternatively, also nucleic acids based on inosine and cytidine as e.g. described in the WO 01/93903, or deoxynucleic acids containing deoxy-inosine and/or deoxyuridine residues (described in WO 01/93905 and PCT/EP 02/05448, incorporated herein by reference) may preferably be used as immunostimulatory nucleic acids for the present invention. Preferably, the mixtures of different immunostimulatory nucleic acids may be used according to the present invention.

It is also within the present invention that any of the aforementioned polycationic compounds is combined with any of the immunostimulatory nucleic acids as aforementioned. Preferably, such combinations are according to the ones as described in WO 01/93905, WO 02/32451, WO 01/54720, WO 01/93903, WO 02/13857 and PCT/EP 02/05448 and the Austrian patent application A 1924/2001, incorporated herein by reference.

In addition or alternatively such vaccine composition may comprise apart from the hyperimmune serum reactive antigens and fragments thereof, and the coding nucleic acid molecules thereof according to the present invention a neuroactive compound. Preferably, the neuroactive compound is human growth factor as, e.g. described in WO 01/24822. Also preferably, the neuroactive compound is combined with any of the polycationic compounds and/or immunostimulatory nucleic acids as afore-mentioned.

In a further aspect the present invention is related to a pharmaceutical composition. Such pharmaceutical composition is, for example, the vaccine described herein. Also a pharmaceutical composition is a pharmaceutical composition which comprises any of the following compounds or combinations thereof: the nucleic acid molecules according to the present invention, the hyperimmune serum reactive antigens and fragments thereof according to the present invention, the vector according to the present invention, the cells according to the present invention, the antibody according to the present invention, the functional nucleic acids according to the present invention and the binding peptides such as the anticalins according to the present invention, any agonists and antagonists screened as described herein. In connection therewith any of these compounds may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a hyperimmune serum reactive antigen and fragments thereof of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

Alternatively the composition may be formulated for topical application, for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1 % to about 98 % by weight of the formulation; more usually they will constitute up to about 80 % by weight of the formulation.

In addition to the therapy described above, the compositions of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.05-5 µg antigen / per kg of body weight, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks.

With the indicated dose range, no adverse toxicological effects should be observed with the compounds of the invention which would preclude their administration to suitable individuals.

In a further embodiment the present invention relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. The ingredient(s) can be present in a useful amount, dosage, formulation or combination. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, reflecting approval by the agency of the manufacture, use or sale of the product for human administration.

In connection with the present invention any disease related use as disclosed herein such as, e. g. use of the pharmaceutical composition or vaccine, is particularly a disease or diseased condition which is caused by, linked or associated with Enterococci, more preferably, *E. faecalis*. In connection therewith it is to be noted that *E. faecalis* comprises several strains including those disclosed herein. A disease related, caused or associated with the bacterial infection to be prevented and/or treated according to the present invention includes besides others bacterial pharyngitis, scarlet fever, impetigo, rheumatic fever, necrotizing fasciitis and sepsis in humans.

In a still further embodiment the present invention is related to a screening method using any of the hyperimmune serum reactive antigens or nucleic acids according to the present invention. Screening methods as such are known to the one skilled in the art and can be designed such that an agonist or an antagonist is screened. Preferably an antagonist is screened which in the present case inhibits or prevents the binding of any hyperimmune serum reactive antigen and fragment thereof according to the present invention to an interaction partner. Such interaction partner can be a naturally occurring interaction partner or a non-naturally occurring interaction partner.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the function of hyperimmune serum reactive antigens and fragments thereof or nucleic acid molecules of the present invention, such as its interaction with a binding molecule. The method of screening may involve high-throughput.

For example, to screen for agonists or antagonists, the interaction partner of the nucleic acid molecule and nucleic acid, respectively, according to the present invention, maybe a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, may be prepared from a cell that expresses a molecule that binds to the hyperimmune serum reactive antigens and fragments thereof of the present invention. The preparation is incubated with labelled hyperimmune serum reactive antigens and fragments thereof in the absence or the presence of a candidate molecule which may be an agonist or antagonist. The ability of the candidate molecule to bind the binding molecule is reflected in decreased binding of the labelled ligand. Molecules which bind gratuitously, i. e., without inducing the functional effects of the hyperimmune serum reactive antigens and fragments thereof, are most likely to be good antagonists. Molecules that bind well and elicit functional effects that are the same as or closely related to the hyperimmune serum reactive antigens and fragments thereof are good agonists.

The functional effects of potential agonists and antagonists may be measured, for instance, by determining the activity of a reporter system following interaction of the candidate molecule with a cell or appropriate cell preparation, and comparing the effect with that of the hyperimmune serum reactive antigens and fragments thereof of the present invention or molecules that elicit the same effects as the hyperimmune serum reactive antigens and fragments thereof. Reporter systems that may be useful in this regard include but are not limited to colorimetric labelled substrate converted into product, a reporter gene that is responsive to changes in the functional activity of the hyperimmune serum reactive antigens and fragments thereof, and binding assays known in the art.

Another example of an assay for antagonists is a competitive assay that combines the hyperimmune serum reactive antigens and fragments thereof of the present invention and a potential antagonist with membrane-bound binding molecules, recombinant binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. The hyperimmune serum reactive antigens and fragments thereof can be labelled such as by radioactivity or a colorimetric compound, such that the molecule number of hyperimmune serum reactive antigens and fragments thereof bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to a hyperimmune serum reactive antigen and fragments thereof of the invention and thereby inhibit or extinguish its activity. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds to the same sites on a binding molecule without inducing functional activity of the hyperimmune serum reactive antigens and fragments thereof of the invention.

Potential antagonists include a small molecule which binds to and occupies the binding site of the hyperimmune serum reactive antigens and fragments thereof thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules.

Other potential antagonists include antisense molecules (see {Okano, H. et al., 1991}; OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION; CRC Press, Boca Raton, FL (1988), for a description of these molecules).

Preferred potential antagonists include derivatives of the hyperimmune serum reactive antigens and fragments thereof of the invention.

As used herein the activity of a hyperimmune serum reactive antigen and fragment thereof according to the present invention is its capability to bind to any of its interaction partner or the extent of such capability to bind to its or any interaction partner.

In a particular aspect, the invention provides the use of the hyperimmune serum reactive antigens and fragments thereof, nucleic acid molecules or inhibitors of the invention to interfere with the initial physical interaction between a pathogen and mammalian host responsible for sequelae of infection. In particular the molecules of the invention may be used: i) in the prevention of adhesion of *E. faecalis* to mammalian extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; ii) to block protein mediated mammalian cell invasion by, for example, initiating phosphorylation of mammalian tyrosine kinases (Rosenshine, I. et al., 1992) to block bacterial adhesion between mammalian extracellular matrix proteins and bacterial proteins which mediate tissue damage; iv) to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

Each of the DNA coding sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein upon expression can be used as a target for the screening of antibacterial drugs. Additionally, the DNA sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The antagonists and agonists may be employed, for instance, to inhibit diseases arising from infection with Enterococci, especially *E. faecalis*, such as sepsis.

In a still further aspect the present invention is related to an affinity device such affinity device comprises at least a support material and any of the hyperimmune serum reactive antigens and fragments thereof according to the present invention, which is attached to the support material. Because of the specificity of the hyperimmune serum reactive antigens and fragments thereof according to the present invention for their target cells or target molecules or their interaction partners, the hyperimmune serum reactive antigens and fragments thereof allow a selective removal of their interaction partner(s) from any kind of sample applied to the support material provided that the conditions for binding are met. The sample may be a biological or medical sample, including but not limited to, fermentation broth, cell debris, cell preparation, tissue preparation, organ preparation, blood, urine, lymph liquid, liquor and the like.

The hyperimmune serum reactive antigens and fragments thereof may be attached to the matrix in a covalent or non-covalent manner. Suitable support material is known to the one skilled in the art and can be selected from the group comprising cellulose, silicon, glass, aluminium, paramagnetic beads, starch and dextrane.

The present invention is further illustrated by the following figures, examples and the sequence listing from which further features, embodiments and advantages may be taken. It is to be understood that the present examples are given by way of illustration only and not by way of limitation of the disclosure.

In connection with the present invention

Figure 1 shows the characterization of *E. faecalis* specific human sera.

Figure 2 shows the characterization of the small fragment genomic library, LEF-70, from *Enterococcus faecalis* V583.

Figure 3 shows the selection of bacterial cells by MACS using biotinylated human IgGs.

Table 1 shows the summary of all screens performed with genomic *E. faecalis* libraries and human serum and *E. faecium* proteins identified by homology search.

Table 2 shows the epitope serology with human sera.

The figures to which it might be referred to in the specification are described in the following in more details.

Figure 1 shows the characterization of human sera for anti-*E. faecalis* antibodies as measured by immune assays. Total anti- *E. faecalis* IgG antibody levels were measured by standard ELISA using total bacterial lysates or culture supernatant fractions prepared from *E. faecalis* strain V583 as coating antigens. (A) Results of representative experiments are shown with healthy adult sera. Data are expressed as ELISA units calculated from absorbance at 405nm at two serum dilutions in the linear range of detection (2,000X and 10,000X). (B) Immunoblot analysis was performed with high titer sera from healthy adults selected by ELISA in order to ensure multiple immune reactivity with protein antigens. Results of a representative experiment using total bacterial lysate and selected human sera at 5,000X dilution are shown. Blots were developed with anti-human IgG secondary antibody reagent. Lanes 1-4: individual high titer sera included in screening pools (N1-4), lane 5: low titer serum (N5), lane 6: negative control, (no serum, 2nd antibody only). Mw: molecular weight markers. (C) shows the comparison of IgG titers obtained with sera from healthy adults (N, numbering is the same as for (B)) vs. acute phase patients (P) with enterococcal infections (such as wound infection, bacteremia, infected catheter). Data are expressed as ELISA units, and were calculated as for (A). (D) shows the results of experiments with convalescent sera from endocarditis patients. Data are expressed as ELISA units calculated from absorbance at 405nm at serum dilution in the linear range of detection (2,000X). (E) Immunoblot analysis was performed with high titer sera from endocarditis patients in order to ensure multiple immune reactivity with protein antigens. Results of a representative experiment using total bacterial lysate and selected human sera at 5,000X dilution are shown. Blots were developed with anti-human IgG secondary antibody reagent. Lanes 1-16: individual sera from endocarditis patients. Mw: molecular weight markers.

Figure 2 (A) shows the fragment size distribution of the *Enterococcus faecalis* V583 small fragment genomic library, LEF-70. After sequencing 576 randomly selected clones, sequences were trimmed to eliminate vector residues and the numbers of clones with various genomic fragment sizes were plotted. (B) shows the graphic illustration of the distribution of the set of 483 randomly sequenced clones of LEF-70 over the *Enterococcus faecalis* V583 chromosome. Rectangles indicate matching sequences to annotated ORFs and diamonds represent fully matched clones to non-coding chromosomal sequences in +/- or +/- orientation, respectively. Circles position all clones with chimeric sequences. Numeric distances in base pairs are indicated over the circular genome for orientation. Partitioning of various clone sets within the library is given in numbers and percentage at the bottom of the figure.

Figure 3A shows the MACS selection with biotinylated human IgGs. The LEF-70 library in pMAL9.1 was screened with 20 µg biotinylated, human serum (IC10-IgG) in the first and in the second selection round. As negative control, no serum was added to the library cells for screening. Number of cells selected after the 1st and 2nd elution for round 1 and 1st to 4th elution for round 2 are shown for the selection with and without added IgGs. Figure 3B shows the reactivity of specific clones (1-26) isolated after 2 rounds of bacterial surface display as analysed by Western blot analysis with the human serum (IC10-IgG, approximately 4 µg/µl) used for selection by MACS at a dilution of 1:3,000. As a loading control the same blot was also analysed with antibodies directed against the platform protein LamB at a dilution of 1:5,000. LB, Extract from a clone expressing LamB without foreign peptide insert.

Table 1: Immunogenic proteins identified by bacterial surface display.

1a shows the antigens identified with sera from healthy adults with high anti-enterococcus titer. A, LEF-300 library from *Enterococcus faecalis* V583 in *fhuA* with IC10-IgG (723), B, LEF-300 library in *fhuA* with IC9-IgG (389), C, LEF-70 library in *lamB* with IC10-IgG (1096), D, LEF-70 library in *lamB* with IC9-IgG (1065). Table 1b shows *E. faecium* proteins identified by homology search with a minimum of 70% identity to *E. faecalis* antigens listed in table 1a. The computer program TBLASTN was used to determine identity between *E. faecalis* and *E. faecium* sequences (<http://www.hgsc.bcm.tmc.edu/microbial/microbialblast.cgi?organism=Efaecium>). The amino acid sequence identity was calculated based on the complete ORF sequence of the corresponding *E. faecalis* antigen. 1c shows the antigens identified with sera from convalescing endocarditis patients with high anti-enterococcus titer. E, LEF-70 library from *Enterococcus faecalis* V583 in *LamB* with P25-IgG (843); F, LEF-70 library in *LamB* with P26-IgG (845); G, LEF-300 library in *FhuA* with P25-IgG (691); H, LEF-300 library in *FhuA* with P26-IgG (770); *, prediction of antigenic sequences longer than 5 amino acids was performed with the program ANTIGENIC [Kolaskar, A. et al., 1990].

Table 2: Epitope serology with human sera.

Immune reactivity of individual synthetic peptides representing selected epitopes with individual human sera is shown. Extent of reactivity is colour coded; white: - (<100 U), light grey: + (100-249 U), dark grey: ++ (250-349 U), black: +++ (>350 U). ELISA units (U) are calculated from OD_{405nm} readings and the serum dilution. Score is calculated as the sum of all reactivities (- = 0; + = 1; ++ = 2; +++ = 3). N1 to N10 sera are high titer sera from healthy adults used in the screens with IC9- and IC10 IgG pools. P1-P11 are sera from patients with *E. faecalis* infections. Location of synthetic peptides within the antigenic ORFs according to the genome annotation of V583 strain are given indicating the first and last amino acid residues. Peptide names: EF0020.1 present in annotated ORF EF0020 on the chromosome; ARF0679.1, potential novel ORF in alternative reading-frame of EF0679, EFC0034.1 present in annotated ORF from plasmid C; ARFC0021.1 present in potential novel ORF in alternative reading-frame of ARFC0021 from plasmid C.

EXAMPLES

Example 1: Characterization and selection of human serum sources based on anti-*E. faecalis* antibodies, preparation of antibody screening reagents

Experimental procedures

Enzyme linked immune assay (ELISA).

ELISA plates (Maxisorb, Millipore) were coated with 5-10 µg/ml total protein diluted in coating buffer (0.1M sodium carbonate pH 9.2). Three dilutions of sera (2,000X, 10,000X, 50,000X) were made in PBS-BSA. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG or anti-human IgA secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000x). Antigen-antibody complexes were quantified by measuring the conversion of the substrate (ABTS) to colored product based on OD_{405nm} readings by automatic ELISA reader (TECAN SUNRISE).

Preparation of bacterial antigen extracts

Total bacterial lysate: Bacteria were grown overnight in BHI (Brain-heart Infusion) and lysed by repeated freeze-thaw cycles: incubation on dry ice/ethanol-mixture until frozen (1 min), then thawed at 37°C (5 min); repeated 3 times. This was followed by sonication and collection of supernatant by centrifugation (3,500 rpm, 15 min, 4°C).

Culture supernatant: After removal of bacteria by centrifugation, the supernatant of overnight grown bacterial cultures was precipitated with ice-cold ethanol by mixing 1 part supernatant with 3 parts absolute ethanol and incubated overnight at -20°C. Precipitates were collected by centrifugation (2,600 g, for 15 min). Dry pellets were dissolved either in PBS for ELISA, or in urea and SDS-sample buffer for SDS-PAGE and immunoblotting. The protein concentration of samples was determined by Bradford assay.

Immunoblotting

Total bacterial lysate and culture supernatant samples were prepared from *in vitro* grown *E. faecalis* strain V583. 10 to 25 µg total protein/lane was separated by SDS-PAGE using the BioRad Mini-Protean Cell electrophoresis system and proteins transferred to nitrocellulose membrane (ECL, Amersham Pharmacia). After overnight blocking in 5% milk, human sera were added at 2,000x dilution, and HRP-labeled anti-human IgG was used for detection.

Purification of antibodies for genomic screening: Five sera from both the patient and the healthy group were selected based on the overall anti-*E. faecalis* titers for serum pools used in the screening procedure. Antibodies against *E. coli* proteins were removed by incubating the heat-inactivated sera with whole cell *E. coli* cells (DH5alpha, transformed with pHIE11, grown under the same condition as used for bacterial surface display). Highly enriched preparations of IgGs from the pooled, depleted sera were generated by protein G affinity chromatography, according to the manufacturer's instructions (UltraLink Immobilized Protein G, Pierce). The efficiency of depletion and purification was checked by SDS-PAGE, Western blotting, ELISA and protein concentration measurements.

Results

The antibodies produced against *E. faecalis* by the human immune system and present in human sera are indicative of the *in vivo* expression of the antigenic proteins and their immunogenicity. These molecules are essential for the identification of individual antigens in the approach as described in the present invention, which is based on the interaction of the specific anti-enterococcal antibodies and the corresponding Enterococcal peptides or proteins. To gain access to relevant antibody repertoires, human sera were collected from healthy adults, as well from patients with enterococcal infections and naïve individuals, young children between 5 and 10 months of age, after they already lost maternal antibodies.

Antibodies in serum and other body fluids, induced in individuals exposed to the pathogens are crucial for antigen identification. The exposure to Enterococci results in asymptomatic colonization, current or past acute or chronic infection. *E. faecalis* colonization and infections are common, and antibodies are present as a consequence of natural immunization from previous encounters. Since Enterococci are opportunistic (not obligate) pathogens, it is likely that sera from healthy individuals also contain relevant antibodies.

70 sera from healthy adults, 16 from patients convalescing from endocarditis caused by *E. faecalis* and 8 from patients with acute infection (mainly wound and bacteremia) were characterized for anti-*E. faecalis* antibodies by a series of immune assays. Primary characterization was done by ELISA using two different antigen preparations, such as total bacterial extract and culture supernatant proteins prepared from *E. faecalis* V583 strain. Representative experiments are shown in Fig. 1 using sera from the healthy adult population (Fig. 1A) and from endocarditis patients (Fig. 1D). Antibody titers were compared at given dilutions where the response was linear. Sera were ranked based on the IgG reactivity against the two complex antigenic mixtures, and the highest ones were selected for further testing by immunoblotting. This analysis confirmed high antibody reactivity of the pre-selected sera against multiple Enterococcal proteins (Fig. 1B&E), especially when compared to not selected, low-titer sera. ELISA ranking of sera did not always correlated with immunoblot signals suggesting that antibodies against non-protein components (e.g. lipoteichoic acid, peptidoglycan, etc.) contributed to the total ELISA

reactivities against total bacterial extracts. Thus the final selection of sera to be included in antibody-pools was based mainly on multiple immunogenic bands in immunoblotting experiments. This extensive antibody characterization approach has led to the unambiguous identification of anti-*E. faecalis* hyperimmune sera. These selected sera from healthy adults were compared to sera from patients with various acute enterococcal infections, such as wound infection, bacteremia and catheter related infections. It was obvious that anti-*E. faecalis* antibody titer was lower in the acute patient group, suggesting that disease might occur in low titer individuals (Fig.1C). These patient sera were then used mainly for validation purposes, that is measuring antibody levels against identified epitopes. However, patients with endocarditis caused by *E. faecalis* (verified by routine microbiological diagnosis) developed high levels of antibodies in the convalescent phase, as it is demonstrated by the high percentage of high titer sera among the 16 samples analysed here (Fig. 1E&D).

10 sera were selected from the healthy adults and 10 from the convalescent patients with endocarditis donor groups. Sera were pooled (5 samples/pool), and IgG purified for antigen identification by bacterial surface display IgG antibodies were purified from pooled sera by affinity chromatography and depleted of *E. coli* -reactive antibodies to avoid background in the bacterial surface display screen (two IgG pools: NEf9, NEf10).

Example 2: Generation of highly random, frame-selected, small-fragment, genomic DNA libraries of *Enterococcus faecalis*

Experimental procedures

Preparation of enterococcal genomic DNA. 50 ml Brain heart infusion (BHI) medium was inoculated with *Enterococcus faecalis* V583 bacteria from a frozen stab and grown with aeration and shaking for 18 h at 37°C. The culture was then harvested, centrifuged with 1,600x g for 15 min and the supernatant was removed. Bacterial pellets were washed 3 x with PBS and carefully re-suspended in 0.5 ml of Lysozyme solution (100 mg/ml). 0.1 ml of 10 mg/ml heat treated RNase A and 20 U of RNase T1 were added, mixed carefully and the solution was incubated for 1 h at 37°C. Following the addition of 0.2 ml of 20 % SDS solution and 0.1 ml of Proteinase K (10 mg/ml) the tube was incubated overnight at 55°C. 1/3 volume of saturated NaCl was then added and the solution was incubated for 20 min at 4°C. The extract was pelleted in a microfuge (13,000 rpm) and the supernatant transferred into a new tube. The solution was extracted with PhOH/CHCl₃/IAA (25:24:1) and with CHCl₃/IAA (24:1). DNA was precipitated at room temperature by adding 0.6x volume of Isopropanol, spooled from the solution with a sterile Pasteur pipette and transferred into tubes containing 80% ice-cold ethanol. DNA was recovered by centrifuging the precipitates with 10-12,000x g, then dried on air and dissolved in ddH₂O.

Preparation of small genomic DNA fragments. Genomic DNA fragments were mechanically sheared into fragments ranging in size between 150 and 300 bp using a cup-horn sonicator (Bandelin Sonoplus UV 2200 sonicator equipped with a BB5 cup horn, 10 sec. pulses at 100 % power output) or into fragments of size between 50 and 70 bp by mild DNase I treatment (Novagen). It was observed that sonication yielded a much tighter fragment size distribution when breaking the DNA into fragments of the 150-300 bp size range. However, despite extensive exposure of the DNA to ultrasonic wave-induced hydromechanical shearing force, subsequent decrease in fragment size could not be efficiently and reproducibly achieved. Therefore, fragments of 50 to 70 bp in size were obtained by mild DNase I treatment using Novagen's shotgun cleavage kit. A 1:20 dilution of DNase I provided with the kit was prepared and the digestion was performed in the presence of MnCl₂ in a 60 µl volume at 20°C for 5 min to ensure double-stranded cleavage by the enzyme. Reactions were stopped with 2 µl of 0.5 M EDTA and the fragmentation efficiency was evaluated on a 2% TAE-agarose gel. This treatment resulted in total fragmentation of genomic DNA into near 50-70 bp fragments. Fragments were then blunt-ended twice using T4 DNA Polymerase in the presence of 100 µM each of dNTPs to ensure efficient flushing of the ends. Fragments

were used immediately in ligation reactions or frozen at -20°C for subsequent use.

Description of the vectors. The vector pMAL4.31 was constructed on a pASK-IBA backbone [Skerra, A., 1994] with the beta-lactamase (*bla*) gene exchanged with the Kanamycin resistance gene. In addition the *bla* gene was cloned into the multiple cloning site. The sequence encoding mature beta-lactamase is preceded by the leader peptide sequence of *ompA* to allow efficient secretion across the cytoplasmic membrane. Furthermore a sequence encoding the first 12 amino acids (spacer sequence) of mature beta-lactamase follows the *ompA* leader peptide sequence to avoid fusion of sequences immediately after the leader peptidase cleavage site, since e.g. clusters of positive charged amino acids in this region would decrease or abolish translocation across the cytoplasmic membrane [Kajava, A. et al., 2000]. A *Sma*I restriction site serves for library insertion. An upstream *Fse*I site and a downstream *Not*I site, which were used for recovery of the selected fragment, flank the *Sma*I site. The three restriction sites are inserted after the sequence encoding the 12 amino acid spacer sequence in such a way that the *bla* gene is transcribed in the -1 reading frame resulting in a stop codon 15 bp after the *Not*I site. A +1 bp insertion restores the *bla* ORF so that beta-lactamase protein is produced with a consequent gain of Ampicillin resistance.

The vector pMAL9.1 was constructed by cloning the *lamB* gene into the multiple cloning site of pEH1 [Hashemzadeh-Bonehi, L. et al., 1998]. Subsequently, a sequence was inserted in *lamB* after amino acid 154, containing the restriction sites *Fse*I, *Sma*I and *Not*I. The reading frame for this insertion was constructed in such a way that transfer of frame-selected DNA fragments excised by digestion with *Fse*I and *Not*I from plasmid pMAL4.31 yields a continuous reading frame of *lamB* and the respective insert.

The vector pHIE11 was constructed by cloning the *fhuA* gene into the multiple cloning site of pEH1. Thereafter, a sequence was inserted in *fhuA* after amino acid 405, containing the restriction site *Fse*I, *Xba*I and *Not*I. The reading frame for this insertion was chosen in a way that transfer of frame-selected DNA fragments excised by digestion with *Fse*I and *Not*I from plasmid pMAL4.31 yields a continuous reading frame of *fhuA* and the respective insert.

Cloning and evaluation of the library for frame selection. Genomic *E. faecalis* DNA fragments were ligated into the *Sma*I site of the vector pMAL4.31. Recombinant DNA was electroporated into DH10B electrocompetent *E. coli* cells (GIBCO BRL) and transformants plated on LB-agar supplemented with Kanamycin (50 µg/ml) and Ampicillin (50 µg/ml). Plates were incubated over night at 37°C and colonies collected for large scale DNA extraction. A representative plate was stored and saved for collecting colonies for colony PCR analysis and large-scale sequencing. A simple colony PCR assay was used to initially determine the rough fragment size distribution as well as insertion efficiency. From sequencing data the precise fragment size was evaluated, junction intactness at the insertion site as well as the frame selection accuracy ($3n+1$ rule).

Cloning and evaluation of the library for bacterial surface display. Genomic DNA fragments were excised from the pMAL4.31 vector, containing the *E. faecalis* library with the restriction enzymes *Fse*I and *Not*I. The entire population of fragments was then transferred into plasmids pMAL9.1 (*LamB*) or pHIE11 (*FhuA*), which have been digested with *Fse*I and *Not*I. Using these two restriction enzymes, which recognise an 8 bp GC rich sequence, the reading frame that was selected in the pMAL4.31 vector is maintained in each of the platform vectors. The plasmid library was then transformed into *E. coli* DH5alpha cells by electroporation. Cells were plated onto large LB-agar plates supplemented with 50 µg/ml Kanamycin and grown over night at 37°C at a density yielding clearly visible single colonies. Cells were then scraped off the surface of these plates, washed with fresh LB medium and stored in aliquots for library screening at -80°C.

Results

Libraries for frame selection. Three libraries (LEF-70 and LEF-300) were generated in the pMAL4.31 vector

with sizes of approximately 70 and 300 bp, respectively. For both library, ligation and subsequent transformation of approximately 1 µg of pMAL4.31 plasmid DNA and 50 ng of fragmented genomic *E. faecalis* DNA yielded approximately 1.5×10^5 to 1×10^6 clones after frame selection. To assess the randomness of the libraries, 576 randomly chosen clones of LEF-70 were sequenced. Of these sequences 483 were successfully trimmed and subjected to further bioinformatic analysis. The results showed that of these clones only very few were present more than once. Furthermore, it was shown that 73% of the clones fell in the size range between 25 and 100 bp and on average the clones had a size of 82 bp (Figure 2). Almost all sequences followed the 3n+1 rule, showing that all clones were properly frame selected.

Bacterial surface display libraries. The display of peptides on the surface of *E. coli* required the transfer of the inserts from the LSPy libraries from the frame selection vector pMAL4.31 to the display plasmids pMAL9.1 (LamB) or pHIE11 (FhuA). Genomic DNA fragments were excised by *FseI* and *NotI* restriction and ligation of 5 ng inserts with 0.1 µg plasmid DNA and subsequent transformation into DH5alpha cells resulted in 7×10^5 to 2×10^6 clones. The clones were scraped off the LB plates and frozen without further amplification. These cells served as libraries for the subsequent screening procedure.

Example 3: Identification of highly immunogenic peptide sequences from *E. faecalis* using bacterial surface displayed genomic libraries and human serum

Experimental procedures

MACS screening. Approximately 2.5×10^8 cells from a given library were grown in 5 ml LB-medium supplemented with 50 µg/ml Kanamycin for 2 h at 37°C. Expression was induced by the addition of 1 mM IPTG for 30 min. Cells were washed twice with fresh LB medium and approximately 2×10^7 cells re-suspended in 100 µl LB medium and transferred to an Eppendorf tube.

20 µg of biotinylated, human IgGs purified from serum was added to the cells and the suspension incubated over night at 4°C with gentle shaking. 900 µl of LB medium was added, the suspension mixed and subsequently centrifuged for 10 min at 6,000 rpm at 4°C. Antibody reacting positive clones were captured with biotinylated anti-human-IgG secondary antibodies. Cells were washed once with 1 ml LB and then re-suspended in 100 µl LB medium. 10 µl of MACS microbeads coupled to streptavidin (Miltenyi Biotech, Germany) were added and the incubation continued for 20 min at 4°C. Thereafter 900 µl of LB medium was added and the MACS microbead cell suspension was loaded onto the equilibrated MS column (Miltenyi Biotech, Germany) which was fixed to the magnet. (The MS columns were equilibrated by washing once with 1 ml 70% EtOH and twice with 2 ml LB medium.)

The column was then washed three times with 3 ml LB medium. After removal of the magnet, cells were eluted by washing with 2 ml LB medium. After washing the column with 3 ml LB medium, the 2 ml eluate was loaded a second time on the same column and the washing and elution process repeated. In some cases the washing and elution process was performed up to four times to increase the specificity of the selection procedure, resulting in a final eluate of 2 ml.

A second round of screening was performed as follows. The cells from the final eluate were collected by centrifugation and re-suspended in 1 ml LB medium supplemented with 50 µg/ml Kanamycin. The culture was incubated at 37°C for 90 min and then induced with 1 mM IPTG for 30 min. Cells were subsequently collected, washed once with 1 ml LB medium and suspended in 10 µl LB medium. For the second round of screening the same amount (20 µg) of human, biotinylated IgGs was added and the suspension incubated over night at 4°C with gentle shaking. All further steps were exactly the same as in the first selection round, except that the number of washing, loading and elution cycles was adapted to each individual screening round. Cells selected after two rounds of selection were plated onto LB-agar plates supplemented with 50 µg/ml Kanamycin and grown over night at 37°C.

Evaluation of selected clones by sequencing and Western blot analysis. Selected clones were grown over night at 37°C in 3 ml LB medium supplemented with 50 µg/ml Kanamycin to prepare plasmid DNA using standard procedures. Sequencing was performed at MWG (Germany) or in collaboration with TIGR (U.S.A.).

For Western blot analysis approximately 10 to 20 µg of total cellular protein was separated by 10% SDS-PAGE and blotted onto HybondC membrane (Amersham Pharmacia Biotech, England). The LamB or FhuA fusion proteins were detected using human serum as the primary antibody at a dilution of approximately 1:3,000 to 1:5,000 and anti-human IgG antibodies coupled to HRP at a dilution of 1:5,000 as secondary antibodies. Detection was performed using the ECL detection kit (Amersham Pharmacia Biotech, England). Alternatively, rabbit anti FhuA or mouse anti LamB antibodies were used as primary antibodies in combination with the respective secondary antibodies coupled to HRP for the detection of the fusion proteins.

Results

Screening of bacterial surface display libraries by magnetic activated cell sorting (MACS) using biotinylated Igs. The libraries LEF-70 in pMAL9.1 and LEF-300 in pHIE11 were screened with pools of biotinylated, human IgGs from sera from healthy individuals (Table 1a) or from sera from convalescent endocarditis patients (Table 1c) (see Example 1: *Preparation of antibodies from human serum*). The selection procedure was performed as described under Experimental procedures. Figure 3A shows a representative example of a screen with the LEF-70 library and IC10-IgGs. As can be seen from the colony count after the first selection cycle from MACS screening, the total number of cells recovered at the end is drastically reduced from 1×10^7 cells to approximately 5×10^4 cells, whereas the selection without antibodies added showed a reduction to about 6×10^3 cells (Figure 3A). For the second round, 2×10^5 cells were loaded on the column and almost all (1×10^5) recovered with IC10-IgG after 4 cycles of loading, washing and elution, while fewer than 100 cells were recovered when no IgGs from human serum were added. This result clearly showed that selection was dependent on *E. faecalis* specific antibodies. Typically, the selection resulted in approximately 30 to 70% of clones to be selected specifically with the applied serum IgGs. For each screen between 400 and 1200 clones were subjected to DNA sequencing and the obtained sequence blasted against the genome of *Enterococcus faecalis* V583. This bioinformatic analysis allowed the evaluation of the screen on the basis of the frequency with which individual clones were selected by the addition of human IgGs. In order to confirm the good performance of the screen, all clones selected more than once were picked and subjected to Western blot analysis with the same, pooled serum IgGs (Figure 3B). The analysis of the more frequently selected clones ($> 5 \times$) revealed that more than 90% of these selected clones showed reactivity with antibodies present in the relevant serum whereas the control strain expressing LamB without a *E. faecalis* specific insert did not react with the same serum IgGs. In general, the clones which were selected with a lower frequency ($< 5 \times$), showed a lower percentage of reactivity with the applied serum IgGs. Colony PCR analysis showed that all selected and tested clones contained an insert in the expected size range.

The sequencing of a large number of randomly picked clones (400 to 1200 per screen) led also to the identification of the gene and the corresponding peptide or protein sequence that was specifically recognized by the human serum IgGs used for screening. The frequency with which a specific clone is selected reflects at least in part the abundance and/or affinity of the specific antibodies in the serum used for selection and recognizing the epitope presented by this clone. In that regard it is striking that clones derived from some ORFs (e.g. EF3060, EFA0042) were picked multiple times and in more than one screen, indicating their highly immunogenic property. Table 1a summarizes the data obtained with four screens performed with sera from healthy adults and Table 1c with four screens with convalescent endocarditis patients sera. The latter table contains only the antigens that were novel relative to the ones listed in Table 1a. 26 antigens were identified by both sera, 71 and 42 unique antigenic ORFs were selected by the sera from healthy adults and patients, respectively.

All clones that are presented in Table 1 have been verified by Western blot analysis using whole cellular extracts from single clones to show the indicated reactivity with the pool of human serum used in the respective screen. As can be seen from Table 1, distinct regions of the identified ORF are identified as immunogenic, since variably sized fragments of the proteins are displayed on the surface by the platform proteins.

It is further worth noticing that many of the genes identified by the bacterial surface display screen encode proteins that are either attached to the surface of *E. faecalis* and/or are secreted. This is in accordance with the expected role of surface attached or secreted proteins in virulence of *E. faecalis*.

Table 1b shows *E. faecium* proteins identified by homology search with a minimum of 70% identity to *E. faecalis* antigens listed in table 1a. The computer program TBLASTN was used to determine identity between *E. faecalis* and *E. faecium* sequences (<http://www.hgsc.bcm.tmc.edu/microbial/microbialblast.cgi?organism=Efaecium>). The amino acid sequence identity was calculated based on the complete ORF sequence of the corresponding *E. faecalis* antigen. This result demonstrated that *E. faecium* contains certain structurally related proteins to the clinically closely related species *E. faecalis*. Since the homologous proteins in *E. faecalis* have been provided as hyperimmune serum reactive antigens in the present invention, the *E. faecium* proteins specified in Table 1b provide the same uses, especially the uses as antigens comprised in pharmaceutical composition e.g. vaccines against *Enterococci* infections.

Example 4: Assessment of the reactivity of highly immunogenic peptide sequences with individual human sera.

Experimental procedures

Peptide synthesis

Peptides were synthesized in small scale (4 mg resin; up to 288 in parallel) using standard F-moc chemistry on a Rink amide resin (PepChem, Tübingen, Germany) using a SyroII synthesizer (MultisynTech, Witten, Germany). After the sequence was assembled, peptides were elongated with Fmoc-epsilon-aminohexanoic acid (as a linker) and biotin (Sigma, St. Louis, MO; activated like a normal amino acid). Peptides were cleaved off the resin with 93% TFA, 5% triethylsilane, and 2% water for one hour. Peptides were dried under vacuum and freeze dried three times from acetonitrile/water (1:1). The presence of the correct mass was verified by mass spectrometry on a Reflex III MALDI-TOF (Bruker, Bremen Germany). The peptides were used without further purification.

Enzyme linked immuno assay (ELISA).

Biotin-labeled peptides (at the N-terminus) were coated on Streptavidin ELISA plates (EXICON) at 10 µg/ml concentration according to the manufacturer's instructions. Highly specific Horse Radish Peroxidase (HRP)-conjugated anti-human IgG secondary antibodies (Southern Biotech) were used according to the manufacturers' recommendations (dilution: 1,000x). Sera were tested at two serum dilutions, 200X and 1,000X. Following manual coating, peptide plates were processed and analyzed by the Gemini 160 ELISA robot (TECAN) with a built-in ELISA reader (GENIOS, TECAN).

Results

Following the bioinformatic analysis of selected clones, corresponding peptides were designed and synthesized. In case of epitopes with more than 26 amino acid residues, overlapping peptides were made. All peptides were synthesized with a N-terminal biotin-tag and used as coating reagents on Streptavidin-

coated ELISA plates.

In order to confirm the immunogenicity of selected epitopes, specific antibody levels were measured by peptide ELISA using individual human sera, which were included in the IC9- and IC10 IgG pools used for their identification in bacterial surface display screens. A summary of serum reactivity of 104 peptides present in 61 *E. faecalis* antigens with ten healthy adult screening sera (N1-N10) and eleven sera from patients with different *E. faecalis* infections are shown (P1-P11). The peptides were compared by the score calculated for each peptide based on the number of positive screening sera and the extent of reactivity. Peptides range from highly and widely reactive to weakly positive ones. Among the highest scoring peptide epitopes which are derived from surface proteins, such as LPXTG cell wall proteins (EF0490.3 and EFA0042.1-2), secreted proteins (EF0360.1 and EF0792.1) and also hypothetical proteins (EF0428.1-2, EF3207.2 and EFC0034.1-2) were found. Interestingly, two prominent epitopes belong to alternative reading frames ARF0679 (40 aa long) and ARF2052 (54 aa long) suggesting that these proteins really exist and are expressed *in vivo* during colonization and/or infection. As it was suggested by lower total *E. faecalis*-antibody levels of the patients' sera relative to the high titer healthy sera (Fig. 1C), peptide reactivities were also lower with the patients' sera.

Table 1a: Immunogenic proteins identified by bacterial surface display.

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
EF0020	PTS system component	4-10,14-21,30-36,59-68,77-82,87-93,96-105,112-121,125-133,135-141,150-162,164-183,192-203,207-213,215-226,228-234,241-247,250-285,302-308	B:1, C:10, D:1	135-148	1, 171
EF0032	membrane protein, putative	15-57,60-73,77-101,108-134,136-177,185-201,203-217,226-240,244-254,272-277,283-288,292-343,354-370,380-398,406-437,439-453,473-490,532-538,584-590,595-601,606-612,664-677,679-704,715-724,731-753,759-772,786-794,814-862	A:1, C:2	657-684	2, 172
EF0062	2,3-cyclic-nucleotide 2-phosphodiesterase, putative	4-9,15-36,41-47,54-60,75-81,114-120,131-146,152-158,174-182,194-202,208-215,218-226,255-271,276-285,290-295,302-311,318-328,330-344,352-359,365-377,388-395,398-405,426-432,439-449,455-500,505-513,531-537,542-552,554-561,587-595,606-612,718-734,763-771,775-782,792-801,805-812,822-828,830-843,849-863,876-894,905-911,919-926,935-947,949-958,968-979,1009-1016,1029-1045,1047-1056,1076-1081,1092-1106,1123-1133,1179-1200,1202-1211,1215-1223,1287-1299,1301-1306	A:3, B:1, C:16, D:1	398-431, 1224-1237	3, 173
EF0149	aggregation substance ASP1	17-47,74-80,90-97,126-133,137-148,167-173,179-185,214-223,250-255,270-283,329-338,342-350,352-358,360-367,372-383,398-404,411-421,426-432,435-446,452-462,472-479,515-521,582-592,611-618,623-629,642-659,666-673,678-689,704-725,732-737,744-757,768-789,824-834,842-849,862-868,877-887,904-916,923-928,941-947,962-974,982-992,1019-1030,1032-1044,1046-1052,1065-1075,1077-1087,1108-1121,1124-1132,1137-1151,1170-1182,1190-1206,1208-1214,1227-1233,1242-1251,1254-1273,1282-1298	A:5, C:2	792-825	4, 174
EF0196	site-specific recombinase family protein	19-31,39-67,82-91,104-110,113-128,149-155,161-181	C:3	137-155	5, 175
EF0253	aldehyde dehydrogenase	6-18,54-63,69-85,110-127,142-156,158-167,169-211,238-246,248-257,276-311,339-349,371-380,385-391,394-403,421-438,451-456,483-489	C:2	449-468	6, 176
EF0270	PTS system component	5-15,24-34,50-56,61-83,98-121,123-136,149-162,166-194,202-215,221-227,229-332,337-360,367-402,404-415,427-433,444-462,471-478,487-498,511-518,521-544,550-563,568-574,580-587,597-607,610-616,624-629	A:1, B:2, C:2, D:2	468-498	7, 177
EF0298	cation-transporting ATPase, E1-E2 family	11-19,32-49,57-63,65-71,80-89,91-133,166-181,183-191,201-230,234-257,264-291,297-303,305-314,316-335,337-354,359-366,368-374,383-388,394-405,408-442,446-470,483-490,499-505,513-538,544-555,557-563,568-590,598-608,617-623,627-636,641-647,667-685,687-693,710-723,733-739,742-754,769-815	D:24	366-388	8, 178
EF0355	endolysin	4-16,30-35,42-53,67-76,82-87,101-108,112-130,132-138,147-152,161-183,187-208,218-225,265-281,295-303,305-317,322-334,338-357,360-368,370-383,387-394,400-419,421-430	A:1, B:3	255-336	9, 179
EF0428	conserved hypothetical protein	19-27,36-47,59-66,76-83,101-112,118-125,142-147,162-180,185-196,225-240,246-263,286-304,314-319,327-333,353-367	A:1, C:23	194-214	10, 180
EF0485 (EF0005)	aggregation substance	14-43,70-76,83-89,111-117,122-128,136-145,163-170,175-182,210-219,246-251,266-279,325-331,338-346,348-354,356-363,368-379,422-428,431-441,450-456,466-473,509-515,532-542,549-556,576-586,605-612,617-623,636-653,660-667,674-686,698-719,726-731,738-745,762-783,818-828,836-843,856-862,871-881,903-910,917-922,935-941,956-968,976-986,1013-1024,1026-1038,1059-1069,1071-1081,1102-1115,1118-1126,1131-1145,1164-1176,1187-1200,1202-1208,1221-1227,1236-1245,1248-1267,1273-1292	C:3, D:4	252-287, 805-844	11, 181

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
EF0490	LPXTG-motif cell wall anchor domain protein	4-18,21-28,37-43,56-70,101-113,131-140,142-150,162-170,172-184,193-204,209-227,233-238,246-264	A:2, C:10	93-168	12, 182
EF0517	2-dehydropanoate 2- reductase, putative	14-20,44-50,61-70,77-96,99-106,129-142,168-181,187-196,205-221,225-241,277-296	C:4	257-281	13, 183
EF0570	Osmosensitive K ⁺ channel sensor histidine kinase,	18-29,43-54,64-76,78-84,88-103,125-149,159-176,198-218,230-242,256-271,279-285,287-293,300-306,325-331,344-351,357-364,371-397,400-414,419-464,485-515,517-526,529-537,548-553,573-580,584-590,603-620,639-661,676-681,687-700,716-761,772-780,785-790,795-803,823-836,848-853	A:2	106-134	14, 184
EF0584	ABC transporter, ATP-binding protein	7-13,19-42,44-51,55-75,87-97,99-110,112-118,129-135,141-156,158-178,213-220,230-286,294-308,323-338,345-352,355-365,370-392,394-419,437-446,454-460,474-497,515-526,528-546,569-575	C:3	128-141	15, 185
EF0668	UDP-N- acetyluramoylalan- yl-D-glutamate-2,6- dianinopimelate ligase	12-20,24-33,45-70,73-84,86-94,103-116,118-124,135-142,163-170,176-200,202-224,226-234,237-248,250-262,265-287,296-307,334-341,347-356,361-369,382-396,405-415,418-427,431-439,443-449,452-461,467-474	D:2	113-146	16, 186
EF0792	permease, putative	13-38,44-50,52-59,66-72,83-94,103-110,116-124,131-137,158-180,199-204,218-233,241-264,269-317,326-342,350-356	C:2	70-86	17, 187
EF0795	conserved hypothetical protein	29-35,49-59,63-84,86-97,103-111,113-126,130-144,150-158,174-198,221-231,250-264,266-273,291-298,310-318	A:1, C:3	70-90	18, 188
EF0799	autolysin	19-25,28-52,60-66,71-76,131-142,149-155,157-178,181-213,218-223,237-242,250-257,260-266,272-279,282-290,321-330,373-385,393-407,441-453,461-475,509-521,529-542,577-589,597-610,643-655,663-677,703-718,729-734	A:8, B:5	358-464, 495-570, 604-685	19, 189
EF0851	hypothetical protein	4-29,51-76,116-136,158-173,179-193,207-215	C:2	86-111	20, 190
EF0861	acetyltransferase, GNAT family	5-23,45-70,79-90,93-107,114-122,142-151	C:3	18-36	21, 191
EF0922	conserved hypothetical protein	9-51,68-120,133-149,158-180,186-206,211-220,222-237,248-293,296-310,317-339	C:2	248-260	22, 192
EF0996	cell division protein FtsA	14-24,44-63,69-98,108-119,123-136,155-161,164-176,180-193,203-208,215-223,239-247,274-281,283-289,296-304,306-313,315-327,331-341,343-353,357-386,392-405	C:1, D:3	205-246	23, 193
EF1012	PTS system component	5-13,16-23,36-42,53-63,70-83,96-102	C:2	14-34	24, 194
EF1026	conserved hypothetical protein	4-13,19-35,49-56,59-76,83-107,121-134,144-153,157-164,166-186,194-202,209-216,231-253,257-264	C:21	98-134	25, 195
EF1032	daunorubicin resistance protein	16-32,38-47,58-68,78-89,98-114,117-123,132-141,146-156,164-170,179-188,196-212,219-230,232-237,244-263,265-274,278-293,297-303,306-326,339-349,352-359,362-367,373-379,384-394,396-406,423-443,451-461,465-484,490-497,504-511,523-533,537-547,550-556,558-566,573-579,586-593,598-609,615-642,647-665,671-686,693-713,723-728	A:2	332-378	26, 196
EF1060	peptide ABC transporter, peptide- binding protein, putative	6-21,34-44,58-64,66-74,79-87,114-127,129-143,154-162,174-189,205-214,241-262,266-273,278-297,319-324,328-338,342-351,390-398,409-415,422-435,458-464,471-477,481-486,506-531,534-540,542-550	A:4	315-389	27, 197
EF1093	LPXTG-motif cell wall anchor domain protein	4-28,39-45,52-58,69-82,93-115,122-128,135-140,146-163,177-192,209-215,221-232,271-284,331-337,341-352,360-378,383-390,392-401,409-422,428-435,462-470,474-480,482-496,531-539,541-549,551-560,562-569,576-582,598-618	C:17	98-127	28, 198
EF1141	MutT/nudix family protein	14-27,33-47,61-79,94-104,119-133	C:2	36-60	29, 199
EF1182	autoinducer-2	11-22,29-40,48-62,68-73,96-106,108-118,125-149	C:3	102-126	30, 200

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	production protein LuxS				
EF1277	transcriptional regulator	4-11,45-55,76-83,86-102,105-112,138-144,147-153	D-9	20-48	31, 201
EF1289	conserved domain protein	12-20,28-56,62-68,72-82,93-99,101-107,120-133,135-145,178-186,208-232,279-292	C-2	36-64	32, 202
EF1386	formate/nitrite transporter family protein	6-14,23-48,65-82,92-134,140-181,188-219,228-238,244-253,255-261	C-6	124-145	33, 203
EF1404	MutS2 family protein	11-25,31-38,53-59,62-71,89-99,125-133,151-157,182-190,195-203,208-215,219-229,249-262,267-275,287-295,298-316,318-325,328-334,344-353,357-363,371-377,385-391,396-415,425-436,438-457,471-485,538-552,554-561,606-625,630-636,646-653,669-679,695-704,706-715,722-747,763-773	C-6	714-738	34, 204
EF1561	shikimate 5- dehydrogenase	10-29,33-45,50-60,70-79,83-95,118-124,136-157,176-184,192-205,207-216,223-234,240-246,258-268,275-283	C-1, D-29	37-56	35, 205
EF1584	cysteine synthase A	4-24,27-38,46-54,66-72,81-97,112-119,128-137,152-157,173-179,185-214,219-225,227-248,262-284,286-295,301-307	C-6	117-134	36, 206
EF1597	catalase	26-43,49-56,60-71,74-82,87-98,110-116,131-146,154-164,169-178,183-189,205-214,241-246,255-268,275-292,305-314,316-323,326-340,346-363,397-402,419-429,440-446,452-461,467-475	C-3	29-66	37, 207
EF1601	PTS system component	7-16,21-39,48-58,61-78,82-89,109-136,138-150,152-176,182-247,255-261,267-332,336-345,347-358,362-368,371-392,394-404,407-472,490-498,505-513,527-544,554-582,603-611,614-620,632-638	A-1, B-2, C-9	500-523	38, 208
EF1624	aldehyde dehydrogenase, putative	24-46,77-83,90-97,99-118,123-166,168-177,204-212,229-239,248-262,273-282,287-293,300-319,321-337,340-352,357-366,391-402,411-428,442-450,464-471,479-489	D-2	19-40	39, 209
EF1646	heat shock protein HslVU, ATPase subunit HslU	9-23,25-34,53-58,70-86,90-97,99-116,118-128,131-141,185-191,228-233,237-253,255-261,264-271,273-280,302-312,319-349,351-359,362-369,376-383,387-394,398-406,419-434	C-3	20-31	40, 210
EF1692	hypothetical protein	15-22,37-43,71-87,105-115,121-127,135-142,152-158	D-4	32-52	41, 211
EF1741	catabolite regulator protein	6-12,18-29,37-47,50-58,65-83,85-91,94-99,108-123,142-150,156-163,183-193,215-222,242-249,252-258,261-270,285-308,318-326	A-4	1-95	42, 212
EF1798	hypothetical protein	9-61,65-133,144-155,166-173,175-221,233-276,278-313,329-368	C-2	210-233	43, 213
EF1817	serine proteinase homolog	11-29,33-39,46-51,65-93,107-113,134-143,147-154,166-177,181-188,214-220,233-243,263-269	A-2, B-1, D-3	112-128	44, 214
EF1823	autolysin	8-46,110-134,155-167,174-183,188-201,210-230,253-258,267-282,289-299,312-319,322-327,330-337,365-381,389-402,405-411,419-425,439-447,465-472,489-512,525-532,540-554,577-589,591-599,605-614,616-624,633-649	D-7	503-529	45, 215
EF1978	DNA-3- methyladenine glycosylase	34-49,64-70,90-118,124-131,141-152,159-165	C-6	112-128	46, 216
EF2005	conserved hypothetical protein	5-15,26-45,55-72,80-85,93-100,121-133,142-148,154-167,198-205,209-215,241-254,260-265,271-279	C-2	244-270	47, 217
EF2052	cell division protein FtsK	4-36,38-54,67-83,122-153,159-178,205-212,232-242,244-253,259-268,281-288,298-309,324-331,334-370,372-381,389-401,403-429,441-450,456-462,465-471,473-479,483-504,508-518,537-543,553-565,578-584,592-609,619-625,658-667,669-679,712-719,722-729,737-744,746-752,758-765	C-6	180-226	48, 218
EF2074	ABC transporter, ATP-binding protein	5-17,23-32,49-56,61-67,76-83,85-103,105-111,120-132,145-171,175-185,191-225,231-246	C-23	99-128	49, 219
EF2266	2-dehydro-3- deoxyphosphoglucon ate aldolase	4-24,28-48,52-58,64-79,87-100,104-120,136-152,159-166	C-4	150-163	50, 220
EF2305	toprim domain	15-27,65-71,77-99,104-121,128-154,183-216,223-229,234-255,277-	C-10	77-97	51, 221

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
	protein	287,296-308			
EF2306	conserved hypothetical protein	8-18,44-76,102-109	C:2	49-57	52, 222
EF2307	conserved hypothetical protein	5-14,28-40,42-51,54-60,77-83,89-100,117-124,146-172,176-204,216-231,237-244,267-278,324-334,342-348,396-401,427-433,438-450,452-457,465-471,473-481,491-500,509-515,523-544,550-556,558-569,589-595,606-618,625-632,640-649,665-671,678-688,691-698,717-723,728-734,781-789,800-805,812-821,833-868,873-879,889-905,929-939,988-998,1046-1061,1073-1079,1089-1096,1115-1124,1132-1140,1172-1196,1220-1226,1231-1249,1269-1277,1287-1301,1307-1330,1350-1361,1369-1378,1387-1412,1414-1420,1422-1439,1484-1491,1513-1529,1552-1561,1576-1583,1606-1613,1617-1640,1647-1654,1665-1679,1686-1698,1709-1727,1736-1743,1750-1757,1771-1790,1801-1807,1817-1823,1831-1842,1859-1868,1870-1882,1884-1891,1900-1906,1909-1914,1929-1935,1952-1960,1974-1988,2002-2011,2032-2063,2071-2081,2116-2124,2139-2147,2149-2159,2163-2190,2209-2215,2245-2253,2282-2287,2331-2342,2360-2370,2379-2393,2402-2408,2414-2421,2423-2430,2433-2439,2442-2450,2472-2478,2485-2493,2495-2503,2506-2512,2547-2554,2558-2564,2615-2625,2637-2652,2692-2698,2700-2706,2711-2723,2731-2740,2748-2753,2756-2762,2765-2772,2781-2798,2810-2824,2844-2852,2885-2899,2912-2922,2937-2944,2947-2970,2988-2998,3016-3025,3032-3037,3062-3071,3129-3148,3156-3161	A: 8, B:1, C: 2,	530-607	53, 223
EF2326	reverse transcriptase	31-36,57-62,79-85,90-96,99-112,120-146,162-185,193-203,208-217,219-226,239-253,283-290,298-304,306-321,340-349,351-361,365-372,386-395,407-438,473-486,537-551,558-568,576-594,598-604	D:40	75-95	54, 224
EF2378	DNA polymerase III, alpha subunit, Gram- positive type	14-19,24-30,34-42,45-52,54-64,66-82,95-105,107-118,126-163,171-177,184-201,210-215,260-269,273-279,288-304,321-327,358-364,370-375,380-387,394-404,407-413,421-431,436-451,465-474,504-511,531-552,578-587,614-626,629-636,638-671,691-715,719-729,733-745,752-759,768-777,785-792,794-802,805-824,844-854,867-880,885-891,893-902,907-924,939-948,955-964,966-975,987-1000,1012-1017,1023-1028,1050-1071,1083-1098,1102-1115,1133-1146,1170-1183,1204-1211,1213-1223,1262-1311,1313-1319,1346-1355,1366-1371,1383-1405,1409-1414	D:38	776-819	55, 225
EF2476	penicillin-binding protein 2, putative	12-27,30-38,54-61,64-74,82-96,103-110,117-125,134-140,147-158,185-201,218-225,232-253,265-280,319-325,350-362,366-372,376-386,464-483,485-490,511-521,531-537,542-559,564-574,593-609,613-619,637-642,668-677	A:3, C:8	195-214	56, 226
EF2556	succinate dehydrogenase/fuma- rate reductase, flavoprotein subunit	4-21,59-67,73-79,84-91,141-151,186-197,203-214,222-227,237-245,255-260,281-292,294-311,336-344,346-355,422-437,459-466,484-491	A:1, C:3	77-109	57, 227
EF2563	conserved hypothetical protein	10-45,52-61,63-70,74-102,112-122,124-132,164-178,181-205,212-240,246-256	C:2	226-247	58, 228
EF2570	xanthine dehydrogenase	38-50,53-63,78-87,89-111,126-152,169-176,179-186,193-228,254-267,275-282,288-304,309-318,325-341,346-353,358-367,384-395,404-427,429-435,456-465,467-501,510-521,523-536,541-548,552-560,563-584,589-595,597-620,625-637,639-645,661-666,712-729,734-741,743-750,775-806,809-816,818-840,842-850	C:3	693-714	59, 229
EF2581	oxidoreductase, pyridine nucleotide- disulfide	5-17,30-37,52-75,77-86,88-107,112-135,151-160,178-222,226-246,263-270,279-294,306-314,327-342,345-352,374-381,389-416,422-429,435-449,453-467,473-500,512-522,524-531,542-549,552-560,565-571,575-586,594-600,613-619,625-633,635-641,647-653,667-674,680-699,711-729,735-741,764-775,781-786,792-798,805-813,817-825,833-842,850-855,860-866,869-	A:1, C:7	533-562	60, 230

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		910,917-930,949-990			
EF2617	ribonuclease R	7-14,39-46,61-74,83-89,93-99,110-121,136-150,172-180,182-200,207-216,223-236,238-251,265-271,280-288,294-309,320-336,339-354,362-377,383-389,401-407,435-441,446-453,460-465,472-487,499-511,518-528,533-540,557-570,572-587,631-637,643-658,663-669,672-678,681-687,695-706,714-728	C:2	118-139	61, 231
EF2682	conserved hypothetical protein	5-19,24-30,56-64,69-79,93-100,102-111,117-123,125-133,174-182,185-199,205-224,268-275,311-336	D:20	102-125	62, 232
EF2703	transcriptional regulator	6-35,39-45,57-62,80-85,92-106,117-122,126-171,214-223,253-260,268-273,285-291,295-306,315-320,325-336,361-366	A:1, B:2, D:12	172-202	63, 233
EF2724	endoglucanase, putative	4-13,24-37,45-51,58-66,84-92,112-121,132-141,151-171,175-195,204-212,222-240,262-268,276-295,305-336,338-348,354-362	C:2	160-183	64, 234
EF2782	galactose-1- phosphate uridylyltransferase	10-16,24-35,41-73,78-104,111-121,124-139,141-148,150-164,196-215,224-241,249-282,299-307,315-357,368-378,393-401	D:2	345-367	65, 235
EF2787	rhodanese family protein	4-32,48-53,61-67,84-104,112-118	D:21	106-130	66, 236
EF2812	hypothetical protein	21-28,31-36,65-81,98-105,115-121,123-131,136-142,155-161,177-190	D:15	201-232	67, 237
EF2858	threonyl-tRNA synthetase	4-15,21-27,33-39,42-56,58-64,68-82,84-90,92-98,113-122,146-162,168-175,177-189,191-203,249-268,279-285,287-304,328-342,349-358,371-378,387-393,404-413,419-425,467-479,487-498,513-524,528-539,541-565,572-579,595-606,626-635,637-642	C:8	612-626	68, 238
EF2893	hypothetical protein	7-13,52-70,76-82,97-106,110-117	D:6	13-45	69, 239
EF2927	hydrolase, haloacid dehalogenase-like family	5-10,12-48,59-64,87-102,107-128,131-140,154-161,165-171,173-215	C:2	54-74	70, 240
EF2951	hypothetical protein	4-11,19-28,34-40,74-81,87-98,126-147,163-171,184-193,205-213	B:1, C:2	49-124	71, 241
EF2961	ribokinase	7-14,23-29,35-40,61-67,99-106,111-122,124-133,135-161,187-206,216-229,236-245,262-268,271-280	C:2	256-273	72, 242
EF2986	ABC transporter, ATP-binding protein	4-13,17-37,47-54,85-99,105-113,120-132,147-166,180-186,192-199,204-216	C:2	127-144	73, 243
EF2987	conserved hypothetical protein	14-27,29-37,52-62,68-76,89-96,117-123,125-131,137-145,166-195,205-212,214-222,228-235,258-264,271-281,288-296,308-324,332-339,355-361,365-371	A:1, C:2	268-293	74, 244
EF3023	polysaccharide lyase, family 8	4-21,30-42,54-60,78-85,90-110,141-147,160-168,176-185,194-206,218-225,230-245,251-261,287-293,295-304,320-326,334-347,351-362,386-402,413-423,427-433,439-453,456-477,480-493,507-513,526-539,574-581,591-598,600-609,614-632,655-665,685-691,703-712,742-747,757-775,797-803,813-819,823-829,880-887,901-906,930-944,948-958,962-968,971-995,1002-1009,1017-1023,1036-1053,1069-1081,1107-1124,1129-1152,1178-1195,1211-1223,1249-1266,1271-1288,1334-1340,1346-1367	A:9, D:6	1-63, 171-189	75, 245
EF3041	peptide ABC transporter, peptide- binding protein	4-22,52-63,70-75,94-104,112-125,133-141,176-199,209-216,244-259,287-299,336-352,366-372,386-399,421-436,444-449,457-466,481-487,506-529,531-540	A:2, B:1	295-378	76, 246
EF3051	hypothetical protein	9-30,43-49,58-75,86-96,119-131,138-147,162-167,181-201,208-214	A:4, C:3	16-121	77, 247
EF3060	secreted antigen SagA, putative	4-27,52-58,80-90,92-100,108-114,118-143,169-176,189-198,247-261,281-287,307-317,323-329,352-363,372-381,396-411,413-426,429-440,442-450,456-461,468-479	A:14, B:6, C:1	1-73	78, 248
EF3073	Signal peptidase I	4-32,47-52,57-63,71-78,92-104,126-142,153-175	B:3, C:3	145-163	79, 249
EF3086	conserved hypothetical protein	17-23,35-41,51-70,73-86,104-125	C:2	105-129	80, 250
EF3096	chromosome segregation SMC protein, putative	25-32,41-50,75-85,87-103,115-122,138-149,164-171,188-210,212-220,224-234,256-273,288-299,304-310,330-336,357-365,382-390,399-405,414-421,440-446,454-461,480-486,502-514,518-540,543-553,561-567,572-580,582-588,595-630,633-651,672-	C:14	909-936, 1001-1031	81, 251

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		681,691-709,760-767,813-832,841-848,852-866,873-893,919-925,927-933,940-955,957-978,984-997,1000-1010,1035-1040,1044-1051,1058-1064,1081-1091,1097-1124,1129-1138,1144-1150,1158-1165,1170-1180			
EF3125	primosomal protein N	4-12,19-26,31-41,49-64,66-86,101-117,119-127,134-142,152-161,163-172,179-188,209-218,234-241,276-291,294-300,307-320,324-341,346-356,373-387,389-397,410-416,418-436,444-454,460-472,481-486,500-507,511-535,541-549,553-559,579-586,602-607,613-620,628-640,654-663,671-678,681-691,709-722,741-754,766-774,778-786,797-803	C:4	212-226	82, 252
EF3177	hypothetical protein	4-10,15-27,34-54,60-73,79-88,101-115,120-136,154-162,167-172,222-240	A:3	126-195	83, 253
EF3183	LPXTG-motif cell wall anchor domain protein	5-16,18-25,29-35,57-63,86-91,107-121,123-131,170-179,185-199,204-226,250-255,262-274,291-296,325-347	A:2, B:1	1-38	84, 254
EF3207	conserved hypothetical protein	7-19,22-34,36-42,48-54,60-66,71-76,104-110,118-133,135-145,158-164,167-174,182-193,196-204,217-229,251-290,293-299,309-315	C:1, D:5	288-318	85, 255
EF3276	conserved hypothetical protein	43-51,55-61,66-73,80-90,103-127,133-142,174-180,185-196,203-210,229-235,239-251,258-266,272-278,289-314,316-326,340-346,355-361	A:1	14-27	86, 256
EF3290	sensor histidine kinase	4-25,27-33,35-41,52-74,76-89,99-124,138-144,146-159,167-182,184-191,193-206,211-223,232-240,249-257,270-279,281-287,293-310,322-341,347-356	C:14	292-322	87, 257
EF3295	hypothetical protein	5-13,28-38,43-60,67-72,98-116,122-134,137-151,167-174,177-195,197-216	B:1, C:4	99-195	88, 258
EF3319	citrate lyase, alpha subunit	15-33,35-42,48-57,62-68,73-91,107-119,121-153,173-194,205-210,223-228,234-241,243-259,275-298,308-315,327-340,342-370,376-391,398-404,410-419	A:1, C:50	71-122	89, 259
EFB0002	transposase	12-39,43-64,87-95,99-105,114-126,128-136,139-147,212-225	A:1, C:3	107-141	90, 260
EFA0041	conserved hypothetical protein	6-33,40-45,60-75,79-86,121-129,131-137,161-167,172-178,186-195,203-212,236-244,257-264,278-294,306-312,345-358,368-381,386-395,404-410,412-418	A:4, B:5, D:3	198-270	91, 261
EFA0042	LPXTG-motif cell wall anchor domain protein	18-31,34-41,50-56,69-83,99-106,129-141,147-153,159-168,170-178,190-198,200-212,221-232,237-255,261-266,274-292	A:3, B:2, C:26	118-216	92, 262
EFA0047	aggregation substance precursor	17-47,61-67,87-93,115-121,126-132,140-148,167-173,179-186,214-223,250-255,264-272,282-294,306-318,338-353,358-377,385-401,414-420,433-441,451-457,470-480,505-511,544-550,571-581,600-607,612-618,631-648,655-662,669-681,693-714,721-726,733-740,757-778,813-823,831-838,851-857,866-876,893-905,912-917,930-936,951-963,971-981,1008-1019,1021-1033,1035-1041,1054-1064,1066-1076,1097-1110,1113-1121,1126-1140,1159-1171,1182-1195,1197-1203,1216-1222,1231-1240,1243-1262,1268-1287	A:4, D:2	738-828	93, 263
EFCC0015	hypothetical protein	19-28,40-46,51-57,68-74,81-87,98-108,111-121	D:3	20-36	94, 264
EFCC0025	hypothetical protein	4-17,19-44,60-69,80-86,110-116	A:1, B:1, C:2	33-60	95, 265
EFCC0034	hypothetical protein	8-16,18-28,42-50,53-75,79-86,94-99,122-128,136-142,149-163,166-173,198-212,254-272,288-295,304-318,324-329,343-348,351-364,367-383,389-395,411-420,427-436	C:10, D:150	11-56	96, 266
ARFC0021.1	conserved domain protein	19-25	D:5	6-24	97, 267
ARFC0021.2	conserved domain protein	6-39,59-68	D:3	44-63	98, 268
ARF0031	hypothetical protein	5-14,21-28,38-53	B:1, C:2	29-41	99, 269
ARF0066	hypothetical protein	4-13,31-41,56-65	C:14	32-56	100, 270

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
ARF0076	hypothetical protein	5-12	A:1, C:8	4-21	101, 271
ARF0180	hypothetical protein	4-18	A:1, C:15	17-32	102, 272
ARF0275	hypothetical protein	4-10,23-33	C:2	14-30	103, 273
ARF0283	hypothetical protein	26-34,44-53	D:2	35-52	104, 274
ARF0375	hypothetical protein	none	C:3	1-19	105, 275
ARF0679	hypothetical protein	4-17,23-30,32-37	D:17	6-23	106, 276
ARF0721	hypothetical protein	5-33,40-58,61-66	C:6	45-66	107, 277
ARF1090	hypothetical protein	15-41,61-67	C:5	41-65	108, 278
ARF1583	hypothetical protein	4-12,16-23,26-37	C:4	10-29	109, 279
ARF2052	hypothetical protein	23-39	C:37	37-55	110, 280
ARF2125	hypothetical protein	12-20	D:8	38-55	111, 281
ARF2307.1	hypothetical protein	22-37	C:4	7-22	112, 282
ARF2307.2	hypothetical protein	none	C:2	3-14	113, 283
ARF2323	hypothetical protein	6-16,43-65,71-76	C:14	17-31	114, 284
ARF2505	hypothetical protein	4-13,27-39,42-69	B:1, C:2	17-32	115, 285
ARF2802	hypothetical protein	4-12,26-39	A:1, B:1, C:3	10-25	116, 286
ARF2902	hypothetical protein	none	C:2	2-31	117, 287
ARF3079	hypothetical protein	6-38, 49-62	D:4	39-55	118, 288
ARF3157	hypothetical protein	4-10,24-30	A:1, C:2	2-19	119, 289
ARF3182	hypothetical protein	12-17,25-46	A:2, C:2	15-30	120, 290
ARF3314	hypothetical protein	4-13	A:2, B:1, C:3, D:1	2-28	121, 291
ARFA0022	hypothetical protein	30-38	C:2	17-45	122, 292
CRF0022	hypothetical protein	24-33,55-61	C:6	31-61	123, 293
CRF0073	hypothetical protein	4-26,34-48	D:2	15-33	124, 294
CRF0096	hypothetical protein	9-15	D:3	1-22	125, 295
CRF0115	hypothetical protein	4-31	C:2	14-33	126, 296
CRF0202	hypothetical protein	5-34,49-55,64-82	C:3	69-83	127, 297
CRF0249	hypothetical protein	33-45	D:2	21-39	128, 298
CRF0258	hypothetical protein	7-14,24-32,42-65,79-86	C:3	50-64	129, 299
CRF0264	hypothetical protein	13-27,33-43,45-62	C:3	12-37	130, 300
CRF0339	hypothetical protein	4-15,17-32	C:3	10-26	131, 301
CRF0399	hypothetical protein	4-9,11-43,45-75	C:4	47-69	132, 302
CRF0682	hypothetical protein	4-18,22-37	C:12	17-34	133, 303
CRF0783	hypothetical protein	4-14	D:6	5-24	134, 304
CRF0801	hypothetical protein	7-33,35-46	C:31	1-19	135, 305
CRF0892	hypothetical protein	13-37,69-75	C:9	51-69	136, 306
CRF1041	hypothetical protein	14-24,26-34,37-49,66-78	C:5	2-25	137, 307
CRF1049	hypothetical protein	17-46,52-57,59-64	C:3	54-68	138, 308
CRF1327	hypothetical protein	4-22	C:2	13-25	139, 309
CRF1593	hypothetical protein	8-40,53-63	D:3	29-50	140, 310
CRF1610	hypothetical protein	16-28	C:2	32-40	141, 311
CRF1732	hypothetical protein	14-20,22-28,39-45	C:5, D:9	2-22	142, 312
CRF1830	hypothetical protein	4-13	C:2	12-31	143, 313
CRF1992	hypothetical protein	15-21	D:11	2-17	144, 314
CRF2074	hypothetical protein	4-17	D:2	20-36	145, 315
CRF2099	hypothetical protein	4-19	C:2	9-18	146, 316
CRF2298	hypothetical protein	4-14	D:2	3-19	147, 317
CRF2318	hypothetical protein	4-21,32-40	C:2	21-39	148, 318
CRF2568	hypothetical protein	4-13	C:2	10-27	149, 319
CRF2573	hypothetical protein	18-31,39-47,75-87,89-98	C:3	79-99	150, 320

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<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
CRF2581	hypothetical protein	15-21	C:12, D:29	9-24	151, 321
CRF2647	hypothetical protein	4-14,18-27,30-53,55-64,68-74,81-98	C:7	22-40	152, 322
CRF2706	hypothetical protein	7-24,44-51	C:2	35-60	153, 323
CRF2751	hypothetical protein	10-47	C:3	23-37	154, 324
CRF2768	hypothetical protein	4-10,12-46	C:3	7-22	155, 325
CRF2778	hypothetical protein	20-27	C:3	1-13	156, 326
CRF2790	hypothetical protein	6-19,41-51	C:12	9-37	157, 327
CRF2899	hypothetical protein	4-9,11-17	C:2	9-23	158, 328
CRF2935	hypothetical protein	4-17,23-38,46-66,68-85	D:26	34-46	159, 329
CRF2966	hypothetical protein	4-18,34-59,75-81	D:31	61-84	160, 330
CRF3074	hypothetical protein	6-17	C:2	7-28	161, 331
CRF3084	hypothetical protein	4-32, 56-61	D:6	35-52	162, 332
CRF3120	hypothetical protein	4-14,27-71,74-88,93-110,115-120,124-130,139-154,161-172	C:4	146-171	163, 333
CRF3276	hypothetical protein	4-21	C:39	3-15	164, 334
CRF3277	hypothetical protein	12-17	C:11	9-26	165, 335
CRF3281	hypothetical protein	10-21,45-58	C:3	51-67	166, 336
CRF3285	hypothetical protein	59-66,68-84	D:2	13-42	167, 337
CRFC0021	hypothetical protein	11-16	C:3	1-16	168, 338
CRFC0046	hypothetical protein	4-19,23-37	C:4	10-30	169, 339
NRF0001	hypothetical protein	19-27,35-46,48-66,82-88,99-105,113-119	C:1	42-59	170, 340

Table 1b. *E. faecium* proteins identified by homology search with identified *E. faecalis* antigens

<i>E. faecium</i>	Putative function (by homology to <i>E. faecalis</i>)	<i>E. faecalis</i> antigenic protein	Seq.ID (DNA, Prot.)	Identity to <i>E. faecalis</i> (%)	Seq.ID (DNA, Prot.)
EFN0001	PTS system component	EF0020	1, 171	78	341, 357
EFN0002	conserved hypothetical protein	EF0428	10, 180	80	342, 358
EFN0003	2-dehydropantoate 2-reductase, putative	EF0517	13, 183	77	343, 359
EFN0004	conserved hypothetical protein	EF0795	18, 188	80	344, 360
EFN0005	cell division protein FtsA	EF0996	23, 193	78	345, 361
EFN0006	PTS system component	EF1012	24, 194	87	346, 362
EFN0007	LPXTG-motif cell wall anchor domain protein	EF1093	28, 198	74	347, 363
EFN0008	MutS2 family protein	EF1404	34, 204	77	348, 364
EFN0009	catabolite regulator protein	EF1741	42, 212	80	349, 365
EFN0010	2-dehydro-3-deoxyphosphogluconate aldolase	EF2266	50, 220	76	350, 366
EFN0011	DNA polymerase III, alpha subunit, Gram-positive type	EF2378	55, 225	82	351, 367
EFN0012	rhodanese family protein	EF2787	66, 236	70	352, 368
EFN0013	threonyl-tRNA synthetase	EF2858	68, 238	89	353, 369
EFN0014	conserved hypothetical protein	EF3207	85, 255	85	354, 370
EFN0015	sensor histidine kinase	EF3290	87, 257	73	355, 371
EFN0016	transposase	EFB0002	90, 260	83	356, 372

Table 1c: Immunogenic proteins identified with sera from endocarditis patients by bacterial surface display.

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
EF0008	single-strand binding protein	4-11, 16-34, 48-55, 67-77, 87-106	E:8	153-183	373, 425
EF0028	PTS system component	22-40, 49-65, 70-91, 95-109, 111-125, 146-207, 209-216, 219-225, 229-244, 251-270, 274-286, 292-309, 316-329, 335-355, 358-370, 376-388, 392-419, 425-430, 435-441, 448-453, 464-478, 486-515	F:1	437-465	374, 426
EF0146	surface exclusion protein Sea1 putative	5-19, 25-31, 43-48, 60-79, 88-100, 105-129, 148-171, 187-193, 243-263, 316-322, 334-340, 345-351, 369-378, 381-391, 399-404, 474-483, 502-517, 525-530, 558-568, 579-596, 622-627, 631-638, 644-651, 653-660, 674-680, 687-693, 721-728, 743-753, 760-775, 788-795, 806-813, 821-828, 835-842, 847-859, 868-887	F:13, H:3	300-347	375, 427
EF0153	LPXTG-motif cell wall anchor domain protein	5-26, 37-44, 89-97, 112-118, 121-128, 138-154, 157-165, 176-181, 188-198, 205-218, 223-243, 247-253, 260-279	H:1	76-155	376, 428
EF0394	44 % homology to secreted antigen SagA <i>e. faecium</i>	4-29, 41-46, 49-68, 82-88, 121-147, 158-164, 187-193, 195-208, 229-236, 244-249, 251-263, 269-275, 307-313, 337-343, 348-381, 392-398, 402-408, 432-438	E:96, G:2	85-117, 194-239	377, 429
EF0443	homology to LysM domain protein	5-12, 14-22, 28-34, 40-46, 70-79, 84-129, 152-165, 174-182	G:2	87-109	378, 430
EF0568	potassium-transporting ATPase subunit B	5-16, 18-52, 54-72, 81-86, 118-126, 136-145, 151-157, 168-180, 209-233, 244-270, 295-302, 315-326, 329-337, 345-352, 364-373, 397-402, 408-418, 424-431, 435-443, 472-480, 483-489, 504-510, 519-527, 549-564, 576-599, 605-637, 641-673	E:2	91-98	379, 431
EF0591	lipoprotein putative	23-36, 42-52, 133-140, 151-157, 242-247, 267-277, 295-301, 320-328, 333-339, 345-352, 365-371, 397-403, 415-428, 456-465, 481-487, 489-495, 508-516, 518-527, 585-592, 606-614, 631-637, 643-658, 665-670, 723-728, 737-744, 752-759, 787-793, 835-841, 873-885, 918-928, 938-945, 951-966, 978-988, 1015-1020, 1030-1036, 1044-1052, 1058-1069, 1071-1079, 1081-1088, 1113-1119, 1125-1138, 1141-1147, 1164-1170, 1172-1177, 1190-1200, 1214-1220, 1230-1236, 1239-1245, 1262-1268, 1270-1275, 1288-1298, 1312-1318, 1328-1334, 1337-1343, 1360-1366, 1368-1373, 1386-1396, 1410-1416, 1426-1432, 1435-1441, 1458-1464, 1466-1471, 1484-1494, 1508-1514, 1524-1530, 1533-1539, 1556-1562	F:3	307-340	380, 432
EF0592	LPXTG-motif cell wall anchor domain protein (repeat domains)	19-25, 35-41, 44-50, 66-72, 74-79, 92-102, 116-122, 132-138, 141-147, 164-170, 172-177, 190-200, 214-220, 230-236, 239-245, 262-268, 270-275, 288-298, 312-318, 328-334, 337-343, 360-366, 368-373, 386-396, 410-416, 426-432, 435-441, 458-464, 466-478, 504-524	H:1	79-148, 177-246, 275-344, 373-442	381, 433
EF0658	hypothetical protein	7-14, 16-23, 33-39, 46-53, 72-79, 92-115, 123-130, 156-175, 179-187, 214-220, 239-246, 266-274, 302-325, 338-354, 360-370, 375-390, 392-401, 421-428, 430-463	F:2	29-58	382, 434
EF0727	conserved hypothetical protein	4-9, 22-39, 58-65, 72-82, 87-92, 99-104, 107-119, 143-166, 171-177, 194-202, 205-213, 220-228, 231-240, 247-263, 309-315, 317-323, 336-343	E:1	294-320	383, 435
EF0775	aggregation substance - chimeric	4-10, 12-18, 24-29, 34-43, 50-65, 70-76, 111-117, 129-138, 152-159, 166-171, 184-195, 200-210, 224-236, 241-251, 274-283, 285-296, 313-319, 332-341, 348-355, 378-386, 410-416, 433-445, 475-482, 523-529, 531-540, 584-596, 626-633, 674-680, 682-688, 738-750, 780-787, 828-834, 836-842, 853-862, 882-887, 893-912	H:2	604-676	384, 436
EF0779	conserved domain protein	15-38, 49-57, 60-99, 103-119, 124-194, 200-206, 215-249, 251-291, 307-313, 315-347, 369-378, 383-390, 393-400, 405-411, 423-435, 440-447, 454-460, 470-486, 490-503, 532-539, 542-	H:2	509-583	385, 437

<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		549, 551-567, 579-592			
EF1091	hypothetical protein	38-44, 47-88, 95-103, 157-172, 235-240, 250-260, 263-276, 294-300, 312-317, 331-337, 369-391, 412-419, 442-448, 453-463, 490-529, 537-555, 571-580, 600-617, 619-627, 642-648, 682-687, 693-700, 716-722, 738-748, 756-763, 779-789, 796-802, 820-828, 833-840, 846-853, 862-872, 880-887, 894-899, 924-937, 957-963, 1006-1012, 1043-1049, 1063-1069, 1076-1097	F:4	124-147	386, 438
EF1323	conserved hypothetical protein authentic point mutation	4-28, 31-49, 60-71, 75-102, 104-114, 134-144, 160-184, 250-257, 277-285, 287-294, 330-338, 345-351, 367-374, 381-388, 393-399, 402-407, 420-426, 443-448, 458-464	F:8	411-436, 454-488	387, 439
EF1355	pyruvate dehydrogenase complex E2	20-27, 45-55, 57-64, 66-77, 98-106, 130-137, 155-165, 167-174, 176-187, 194-203, 208-223, 227-238, 245-251, 257-270, 273-278, 287-299, 330-345, 352-358, 363-385, 392-399, 410-417, 437-443, 467-484, 486-492, 495-500, 504-516, 526-536	E:2	219-270	388, 440
EF1699	transcriptional regulator MerR family	11-22, 24-31, 46-63, 65-71, 73-88, 95-109, 174-181, 183-201, 204-212, 216-222, 228-233, 241-247	H:2	142-221	389, 441
EF1744	general stress protein	8-28, 51-59, 67-84, 93-98, 140-152, 154-162, 183-188	F:3	91-125	390, 442
EF1752	conserved hypothetical protein	10-22, 27-61	F:6	69-100	391, 443
EF1753	conserved hypothetical protein	7-15, 18-26, 94-100, 126-131, 152-165, 219-228, 254-263, 274-292, 297-308, 333-340, 342-352, 354-371, 373-379, 403-410, 420-438, 450-456, 463-470, 489-495, 503-512	G:2	97-173	392, 444
EF1791	peptide ABC transporter peptide-binding protein	4-21, 37-43, 49-65, 67-74, 76-90, 113-119, 131-141, 155-173, 175-189, 192-199, 207-221, 247-254, 266-276, 317-322, 337-343, 387-393, 408-428, 439-448, 451-460, 469-479, 482-487, 493-501, 517-523, 533-542	F:1	480-503	393, 445
EF1800	conserved hypothetical protein	11-26, 40-46, 78-86, 93-103, 121-126, 132-138, 166-177, 183-196, 203-212, 214-221, 228-263, 304-311, 323-338, 345-351, 357-363, 379-393, 420-434, 442-448, 518-527, 547-553, 581-591, 602-609, 637-645, 665-674, 687-692, 701-708, 730-739, 796-802, 844-857, 882-888, 903-914, 944-950, 976-983, 1027-1033, 1049-1057, 1066-1072, 1085-1092, 1120-1127, 1137-1144, 1153-1158, 1165-1176, 1181-1187, 1221-1230, 1238-1244, 1269-1274	E:1	605-632	394, 446
EF1818	gelatinase	6-47, 57-65, 83-95, 109-121, 138-147, 154-164, 167-177, 194-200, 202-212, 227-234, 240-253, 260-267, 283-291, 320-329, 340-347, 356-364, 412-422, 430-436, 441-459, 465-475, 478-486, 498-507	F:14	59-84	395, 447
EF1850	conserved hypothetical protein	10-21, 58-83, 88-97, 120-126	F:1	21-51	396, 448
EF1877	conserved hypothetical protein	5-39, 56-62, 76-88, 90-114, 138-162, 170-195, 202-221, 228-250, 264-270, 304-355, 374-387, 391-416, 462-471, 526-546, 554-561, 574-579, 639-645, 651-660, 674-682, 689-694	E:10	666-697	397, 449
EF2174	hypothetical protein	6-30, 36-42, 143-157, 176-197, 202-209, 216-233, 241-246, 275-287, 292-299, 315-325, 343-350, 375-380, 397-403, 411-420, 422-434, 441-448, 467-474, 477-499, 555-568, 591-597, 601-609, 623-644, 667-688, 692-698, 703-718, 736-747, 757-766, 782-791, 795-801, 832-840, 859-865	F:119	226-269	398, 450
EF2224	LPXTG-motif cell wall anchor domain protein	6-23, 43-51, 61-67, 73-82, 91-97, 123-130, 149-158, 164-175, 228-234, 240-246, 248-255, 262-272, 326-332, 340-347, 365-371, 377-388, 409-419, 425-431, 438-445, 449-457, 464-470, 496-507, 559-568, 575-581, 603-608, 617-623, 633-639, 648-654, 659-670, 695-701, 734-752, 806-814, 816-829, 861-868, 891-899, 904-909, 937-945, 947-960, 978-983, 992-999, 1022-1031, 1068-1076, 1078-1091, 1109-1114, 1123-1130, 1153-1162, 1199-1207, 1209-1222, 1254-1261, 1284-1293, 1330-1338, 1340-1353, 1371-1376, 1385-1392, 1415-1421, 1433-	F:2	1422-1458	399, 451

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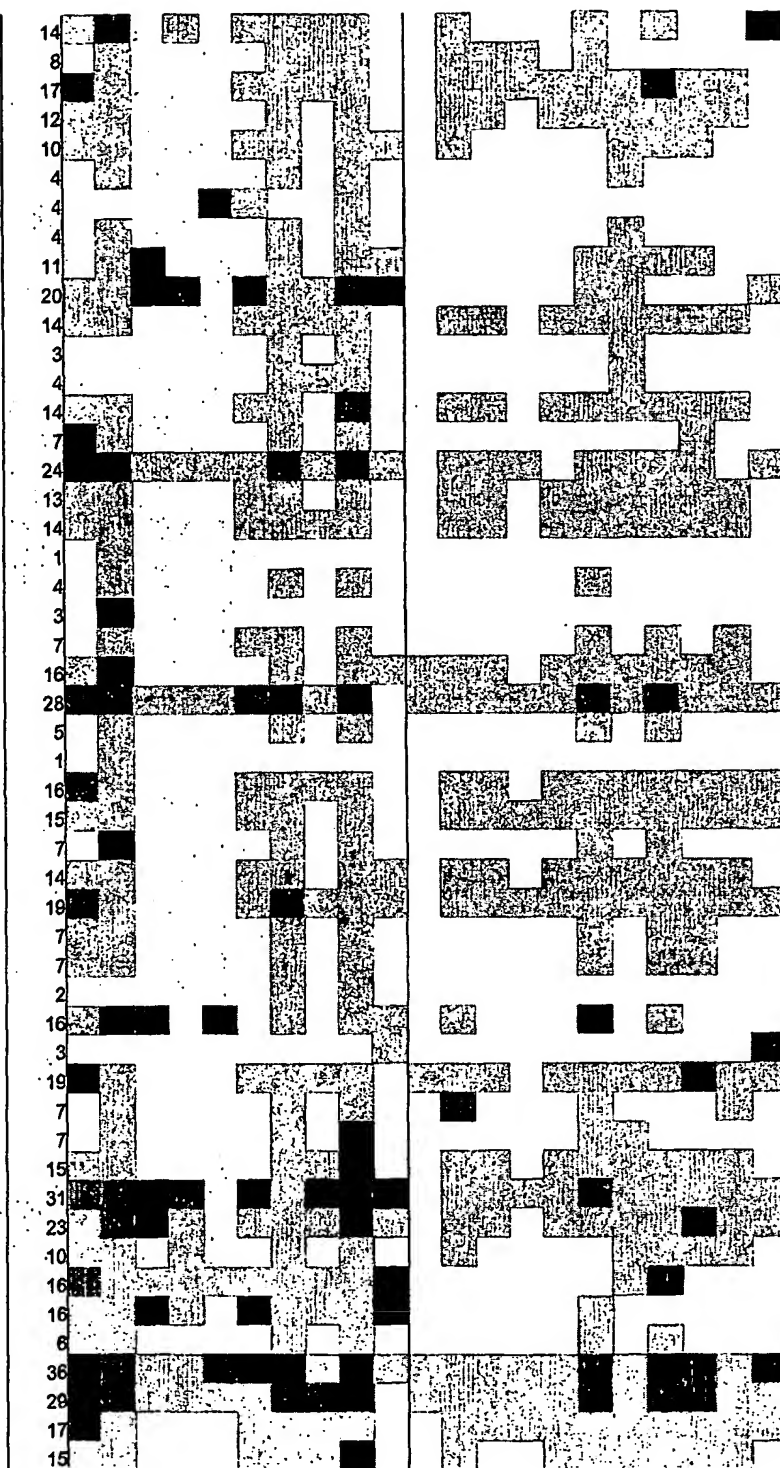
<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
		1438, 1460-1465, 1470-1492			
EF2318	Peptidase M23/M37 family V	82-94, 111-118, 125-131, 206-212, 261-266, 310-320, 328-338, 345-351, 353-360, 414-420, 424-434, 440-447, 451-500, 506-516, 548-561, 566-572, 584-591, 601-622, 630-636, 650-659, 661-667, 674-699, 703-711, 717-729, 736-744, 752-759, 765-771, 813-822, 826-842, 852-868, 870-877, 881-895, 897-906, 913-922	H:5	602-671	400, 452
EF2704	A/G-specific adenine glycosylase	12-18, 20-25, 43-54, 56-65, 73-79, 82-88, 99-111, 136-142, 153-169, 171-183, 195-223, 229-248, 255-260, 272-277, 281-292, 298-319, 322-329, 332-351, 363-379, 381-389	F:5	275-304	401, 453
EF2713	LPXTG-motif cell wall anchor domain protein	4-9, 34-48, 65-77, 101-106, 111-131, 138-153, 186-191, 230-250	H:1	148-219	402, 454
EF2802	endolysin	4-23, 30-35, 42-53, 67-76, 82-87, 101-108, 112-130, 132-138, 147-152, 161-183, 187-208, 218-225, 265-283, 295-303, 306-317, 322-334, 338-357, 360-368, 370-383, 387-398, 400-419, 421-430	H:2	104-182, 240-304	403, 455
EF2813	conserved hypothetical protein	4-12, 63-69, 94-102, 146-164, 166-173, 175-181, 193-207, 263-281, 286-295, 301-306, 330-343, 369-378, 382-388, 414-420, 422-430, 438-454, 456-462, 472-531, 543-560, 581-591, 596-605, 614-623, 626-635, 656-662, 669-676, 683-690, 693-698, 705-711, 728-736, 752-764	E:4	69-102	404, 456
EF2820	hypothetical protein	6-12, 43-53, 141-147, 164-179, 185-195, 197-206, 227-235, 237-271, 288-305, 308-317, 335-341, 351-357, 365-376, 386-395, 397-416, 422-447	E:1	11-35	405, 457
EF3082	iron compound ABC transporter substrate- binding protein	16-24, 50-65, 73-84, 88-99, 114-124, 130-146, 181-187, 193-203, 214-220, 236-247, 250-258, 287-297	F:92	50-113	406, 458
EF3256	lipoprotein putative	4-25, 50-55, 76-82, 117-123, 131-137, 139-148, 157-166, 239-245, 253-258, 266-275, 277-292, 300-306	F:5, G:3	51-83, 93-161	407, 459
EFA0021	hypothetical protein	6-22, 34-43, 51-86, 93-100, 110-116, 150-161, 164-171, 180-187, 197-218	H:2	168-237	408, 460
EFA0044	hypothetical protein	4-27, 55-60, 74-82	E:6	10-46	409, 461
EFA0052	surface exclusion protein Sea1	6-19, 25-31, 43-49, 60-79, 88-100, 105-129, 148-161, 164-171, 187-193, 243-263, 316-322, 334-340, 369-378, 381-391, 398-404, 460-466, 474-483, 502-509, 511-517, 525-530, 558-567, 579-596, 622-627, 631-638, 641-651, 653-659, 674-680, 687-693, 710-716, 720-727, 743-753, 760-775, 788-795, 806-813, 821-828, 836-842, 847-860, 865-880	H:10	258-377	410, 462
EFC0004	TraC protein	4-11, 25-64, 71-79, 88-94, 107-120, 123-132, 167-188, 231-237, 240-246, 261-267, 306-311, 330-342, 351-358, 389-395, 406-418, 429-434, 439-448, 483-501, 511-520	G:1	71-143	411, 463
EFC0012	LPXTG-motif cell wall anchor domain protein	4-18, 22-27, 53-64, 94-100, 121-127, 133-139, 155-164, 177-182, 187-196, 206-218, 224-242, 248-253, 258-277	H:1	184-253	412, 464
EFC0021	conserved domain protein	10-17, 56-67, 72-82, 94-99, 106-113, 166-173, 229-235, 243-283, 295-301, 313-321, 326-331, 342-348, 396-414, 423-435, 446-452, 454-462, 496-502, 511-534, 543-556, 563-570, 586-593, 616-626, 638-645, 653-662, 679-696, 731-737, 766-774, 776-782, 790-796, 810-817, 825-835, 837-846	H:5	540-615	413, 465
EFC0053	Transposase Mutator family	13-24, 30-36, 73-81, 89-95, 109-115, 117-143, 161-173, 179-189, 226-244, 251-261, 275-281, 298-305, 307-315, 323-328, 364-374	G:6, H:12	69-186, 264-354	414, 466
ARFC0021.1	hypothetical protein	19-25	D:5	6-22	415, 467
ARFC0021.2	hypothetical protein	6-39, 59-68	D:3	43-62	416, 468
ARF0324	hypothetical protein	6-14, 22-32	F:2	1-27	417, 469
ARF1627	hypothetical protein	4-41	E:127	28-40	418, 470
ARF1650	hypothetical protein	8-14	F:5	4-19	419, 471
CRF0097	hypothetical protein	4-10, 12-22, 30-35	E:78	6-33	420, 472

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<i>E. faecalis</i> antigenic protein	Putative function (by homology)	predicted immunogenic aa*	No. of selected clones per ORF and screen	Location of identified immunogenic region (aa)	Seq. ID (DNA, Prot.)
CRF0257	hypothetical protein	4-16, 24-33	F:21	37-54	421, 473
CRF0635	hypothetical protein	none	E:7	2-23	422, 474
CRF1152	hypothetical protein	4-21, 27-33, 36-41	E:16	14-34	423, 475
CRF1720	hypothetical protein	4-14, 24-30, 37-42, 57-78, 83-89, 94-103, 113-131	E:5	100-122	424, 476

[illegible]

EF1978.1	216	112-128
EF2052.1	218	181-199
EF2074.1	219	109-121
EF2266.1	220	150-163
EF2305.1	221	77-97
EF2307.1	223	564-586
EF2326.1	224	75-94
EF2378.1	225	776-798
EF2378.2	225	784-808
EF2378.3	225	794-815
EF2476.1	226	196-212
EF2556.1	226	78-100
EF2556.2	226	85-107
EF2581.1	230	536-553
EF2682.1	232	102-125
EF2703.1	233	178-198
EF2858.1	238	612-626
EF3023.1	245	171-187
EF3041.1	246	296-320
EF3041.2	246	315-339
EF3041.3	246	334-358
EF3041.4	246	353-377
EF3051.1	247	47-71
EF3060.1	248	1-25
EF3060.2	248	20-45
EF3060.3	248	40-64
EF3073.1	249	146-161
EF3096.1	251	910-935
EF3096.2	251	1007-1030
EF3125.1	252	212-226
EF3177.1	253	126-152
EF3177.2	253	148-173
EF3177.3	253	169-195
EF3207.1	255	288-310
EF3207.2	255	293-316
EF3290.1	257	293-312
EF3295.1	258	154-170
EF3319.1	259	72-95
EF3319.2	259	90-112
EF3319.3	259	97-121
EFA0042.1	262	135-150
EFA0042.2	262	146-163
EFA0047.1	263	799-827
EFC0034.1	266	23-43
EFC0034.2	266	33-53
ARFC0021.1	268	44-62
ARF0679.1	276	6-22
ARF2052.1	280	37-54
ARF2125.1	281	40-54
ARF2307.1	282	7-21



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Claims:

1. An isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence which is selected from the group consisting of:
 - a) a nucleic acid molecule having at least 70% sequence identity to a nucleic acid molecule selected from Seq ID No 1-2, 4-8, 10, 12-18, 20-23, 25-26, 29-43, 45-62, 64-74, 76-77, 79-83, 85-89, 91-92, 94-114, 117-126, 128-146, 148-170, 373, 375, 379-381, 387, 392, 394, 397-399, 407-408, 410-411 and 415-424,
 - b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
 - c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
 - d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b), or c)
 - e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid molecule defined in a), b), c) or d).
2. The isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 80%, preferably at least 95%, especially 100%.
3. An isolated nucleic acid molecule encoding a hyperimmune serum reactive antigen or a fragment thereof comprising a nucleic acid sequence selected from the group consisting of
 - a) a nucleic acid molecule having at least 96%, preferably at least 98 %, especially 100 % sequence identity to a nucleic acid molecule selected from Seq ID No 3, 9, 11, 24, 27, 44, 63, 75, 84, 115-116, 127, 374, 376-378, 382-386, 388-391, 393, 395-396, 400-406, 409 and 412-414.
 - b) a nucleic acid molecule which is complementary to the nucleic acid molecule of a),
 - c) a nucleic acid molecule comprising at least 15 sequential bases of the nucleic acid molecule of a) or b)
 - d) a nucleic acid molecule which anneals under stringent hybridisation conditions to the nucleic acid molecule of a), b) or c),
 - e) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).
4. An isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of
 - a) a nucleic acid molecule selected from Seq ID No 90, 147,
 - b) a nucleic acid molecule which is complementary to the nucleic acid of a),
 - c) a nucleic acid molecule which, but for the degeneracy of the genetic code, would hybridise to the nucleic acid defined in a), b), c) or d).
5. The nucleic acid molecule according to any one of the claims 1, 2, 3 or 4, wherein the nucleic acid is DNA.
6. The nucleic acid molecule according to any one of the claims 1,2, 3, 4, or 5 wherein the nucleic acid is RNA.
7. An isolated nucleic acid molecule according to any one of claims 1 to 5, wherein the nucleic acid molecule is isolated from a genomic DNA, especially from a *E. faecalis* genomic DNA.
8. A vector comprising a nucleic acid molecule according to any one of claims 1 to 7.
9. A vector according to claim 8, wherein the vector is adapted for recombinant expression of the hyperimmune serum reactive antigens or fragment thereof encoded by the nucleic acid molecule

according to any one of claims 1 to 7.

10. A host cell comprising the vector according to claim 8 or 9.
11. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 1, 2, 5, 6 or 7 and fragments thereof, wherein the amino acid sequence is selected from the group consisting of Seq ID No 171-172, 174-178, 180, 182-188, 190-193, 195-196, 199-213, 215-232, 234-244, 246-247, 249-253, 255-259, 261-262, 264-284, 287-296, 298-316, 318-340, 425, 427, 431-433, 439, 444, 446, 449-451, 459-460, 462-463 and 467-476.
12. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 3, 5, 6, or 7 and fragments thereof, wherein the amino acid sequence is selected from the group consisting of Seq ID No 173, 179, 181, 194, 197, 214, 233, 245, 254, 285-286, 297, 426, 428-430, 434-438, 440-443, 445, 447-448, 452-458, 461 and 464-466.
13. A hyperimmune serum-reactive antigen comprising an amino acid sequence being encoded by a nucleic acid molecule according to any one of the claims 4, 5, 6, or 7 and fragments thereof, wherein the amino acid sequence is selected from the group consisting of Seq ID No 260, 317.
14. Fragments of hyperimmune serum-reactive antigens selected from the group consisting of peptides comprising amino acid sequences of column "predicted immunogenic aa" and "location of identified immunogenic region" of Table 1a and Table 1c; the serum reactive epitopes of Table 2, especially peptides comprising amino acid 4-10, 14-21, 30-36, 59-68, 77-82, 87-93, 96-105, 112-121, 125-133, 135-141, 150-162, 164-183, 192-203, 207-213, 215-226, 228-234, 241-247, 250-285, 302-308 and 135-148 of Seq ID No 171; 15-57, 60-73, 77-101, 108-134, 136-177, 185-201, 203-217, 226-240, 244-254, 272-277, 283-288, 292-343, 354-370, 380-398, 406-437, 439-453, 473-490, 532-538, 584-590, 595-601, 606-612, 664-677, 679-704, 715-724, 731-753, 759-772, 786-794, 814-862 and 657-684 of Seq ID No 172; 4-9, 15-36, 41-47, 54-60, 75-81, 114-120, 131-146, 152-158, 174-182, 194-202, 208-215, 218-226, 255-271, 276-285, 290-295, 302-311, 318-328, 330-344, 352-359, 365-377, 388-395, 398-405, 426-432, 439-449, 455-500, 505-513, 531-537, 542-552, 554-561, 587-595, 606-612, 718-734, 763-771, 775-782, 792-801, 805-812, 822-828, 830-843, 849-863, 876-894, 905-911, 919-926, 935-947, 949-958, 968-979, 1009-1016, 1029-1045, 1047-1056, 1076-1081, 1092-1106, 1123-1133, 1179-1200, 1202-1211, 1215-1223, 1287-1299, 1301-1306, 398-431 and 1224-1237 of Seq ID No 173; 17-47, 74-80, 90-97, 126-133, 137-148, 167-173, 179-185, 214-223, 250-255, 270-283, 329-338, 342-350, 352-358, 360-367, 372-383, 398-404, 411-421, 426-432, 435-446, 452-462, 472-479, 515-521, 582-592, 611-618, 623-629, 642-659, 666-673, 678-689, 704-725, 732-737, 744-757, 768-789, 824-834, 842-849, 862-868, 877-887, 904-916, 923-928, 941-947, 962-974, 982-992, 1019-1030, 1032-1044, 1046-1052, 1065-1075, 1077-1087, 1108-1121, 1124-1132, 1137-1151, 1170-1182, 1190-1206, 1208-1214, 1227-1233, 1242-1251, 1254-1273, 1282-1298 and 792-825 of Seq ID No 174; 19-31, 39-67, 82-91, 104-110, 113-128, 149-155, 161-181 and 137-155 of Seq ID No 175; 6-18, 54-63, 69-85, 110-127, 142-156, 158-167, 169-211, 238-246, 248-257, 276-311, 339-349, 371-380, 385-391, 394-403, 421-438, 451-456, 483-489 and 449-468 of Seq ID No 176; 5-15, 24-34, 50-56, 61-83, 98-121, 123-136, 149-162, 166-194, 202-215, 221-227, 229-332, 337-360, 367-402, 404-415, 427-433, 444-462, 471-478, 487-498, 511-518, 521-544, 550-563, 568-574, 580-587, 597-607, 610-616, 624-629 and 468-498 of Seq ID No 177; 11-19, 32-49, 57-63, 65-71, 80-89, 91-133, 166-181, 183-191, 201-230, 234-257, 264-291, 297-303, 305-314, 316-335, 337-354, 359-366, 368-374, 383-388, 394-405, 408-442, 446-470, 483-490, 499-505, 513-538, 544-555, 557-563, 568-590, 598-608, 617-623, 627-636, 641-647, 667-685, 687-693, 710-723, 733-739, 742-754, 769-815 and 366-388 of Seq ID No 178; 4-16, 30-35, 42-53, 67-76, 82-87, 101-108, 112-130, 132-138, 147-152, 161-183, 187-208, 218-225, 265-281, 295-303, 305-317, 322-334, 338-357, 360-368, 370-383, 387-394, 400-419, 421-430 and 255-336 of Seq ID No 179; 19-

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15. A process for producing a *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof according to any one of the claims 11 to 14 comprising expressing the nucleic acid molecule according to any one of claims 1 to 7.
16. A process for producing a cell, which expresses a *E. faecalis* hyperimmune serum reactive antigen or a fragment thereof according to any one of the claims 11 to 14 comprising transforming or transfecting a suitable host cell with the vector according to claim 8 or claim 9.
17. A pharmaceutical composition, especially a vaccine, comprising a hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of claims 11 to 14 or a nucleic acid molecule according to any one of claims 1 to 7.
18. A pharmaceutical composition, especially a vaccine, according to claim 17, characterized in that it further comprises an immunostimulatory substance, preferably selected from the group comprising polycationic polymers, especially polycationic peptides, immunostimulatory deoxynucleotides (ODNs), peptides containing at least two LysLeuLys motifs, neuroactive compounds, especially human growth hormone, alum, Freund's complete or incomplete adjuvants or combinations thereof.
19. Use of a nucleic acid molecule according to any one of claims 1 to 7 or a hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 for the manufacture of a pharmaceutical preparation, especially for the manufacture of a vaccine against enterococcal infection.
20. An antibody, or at least an effective part thereof, which binds at least to a selective part of the hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14.
21. An antibody according to claim 20, wherein the antibody is a monoclonal antibody.
22. An antibody according to claim 20 or 21, wherein said effective part comprises Fab fragments.
23. An antibody according to any one of claims 20 to 22, wherein the antibody is a chimeric antibody.
24. An antibody according to any one of claims 20 to 23, wherein the antibody is a humanized antibody.

25. A hybridoma cell line, which produces an antibody according to any one of claims 20 to 24.
26. A method for producing an antibody according to claim 20, characterized by the following steps:
- initiating an immune response in a non-human animal by administering an hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of the claims 11 to 14, to said animal,
 - removing an antibody containing body fluid from said animal, and
 - producing the antibody by subjecting said antibody containing body fluid to further purification steps.
27. Method for producing an antibody according to claim 21, characterized by the following steps:
- initiating an immune response in a non-human animal by administering an hyperimmune serum-reactive antigen or a fragment thereof, as defined in any one of the claims 12 to 15, to said animal,
 - removing the spleen or spleen cells from said animal,
 - producing hybridoma cells of said spleen or spleen cells,
 - selecting and cloning hybridoma cells specific for said hyperimmune serum-reactive antigens or a fragment thereof,
 - producing the antibody by cultivation of said cloned hybridoma cells and optionally further purification steps.
28. Use of the antibodies according to any one of claims 20 to 24 for the preparation of a medicament for treating or preventing enterococcal infections.
29. An antagonist which binds to the hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14.
30. A method for identifying an antagonist capable of binding to the hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 comprising:
- a) contacting an isolated or immobilized hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14 with a candidate antagonist under conditions to permit binding of said candidate antagonist to said hyperimmune serum-reactive antigen or fragment, in the presence of a component capable of providing a detectable signal in response to the binding of the candidate antagonist to said hyperimmune serum reactive antigen or fragment thereof; and
 - b) detecting the presence or absence of a signal generated in response to the binding of the antagonist to the hyperimmune serum reactive antigen or the fragment thereof.
31. A method for identifying an antagonist capable of reducing or inhibiting the interaction activity of a hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14 to its interaction partner comprising:
- a) providing a hyperimmune serum reactive antigen or a hyperimmune fragment thereof according to any one of claims 11-14,
 - b) providing an interaction partner to said hyperimmune serum reactive antigen or a fragment thereof, especially an antibody according to any one of the claims 20 to 24,
 - c) allowing interaction of said hyperimmune serum reactive antigen or fragment thereof to said interaction partner to form a interaction complex,
 - d) providing a candidate antagonist,
 - e) allowing a competition reaction to occur between the candidate antagonist and the interaction complex,
 - f) determining whether the candidate antagonist inhibits or reduces the interaction activities of the hyperimmune serum reactive antigen or the fragment thereof with the interaction partner.

32. Use of any of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14 for the isolation and/or purification and/or identification of an interaction partner of said hyperimmune serum reactive antigen or fragment thereof.
33. A process for *in vitro* diagnosing a disease related to expression of the hyperimmune serum-reactive antigen or a fragment thereof according to any one of claims 11 to 14 comprising determining the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen and fragment according to any one of claims 1 to 7 or the presence of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11-14.
34. A process for *in vitro* diagnosis of a bacterial infection, especially a enterococcal infection, comprising analysing for the presence of a nucleic acid sequence encoding said hyperimmune serum reactive antigen and fragment according to any one of claims 1 to 7 or the presence of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14.
35. Use of the hyperimmune serum reactive antigen or fragment thereof according to any one of claims 11 to 14 for the generation of a peptide binding to said hyperimmune serum reactive antigen or fragment thereof, wherein the peptide is selected from the group comprising anticalines.
36. Use of the hyperimmune serum-reactive antigen or fragment thereof according to any one of claims 11 to 14 for the manufacture of a functional nucleic acid, wherein the functional nucleic acid is selected from the group comprising aptamers and spiegelmers.
37. Use of a nucleic acid molecule according to any one of claims 11 to 14 for the manufacture of a functional ribonucleic acid, wherein the functional ribonucleic acid is selected from the group comprising ribozymes, antisense nucleic acids and siRNA.

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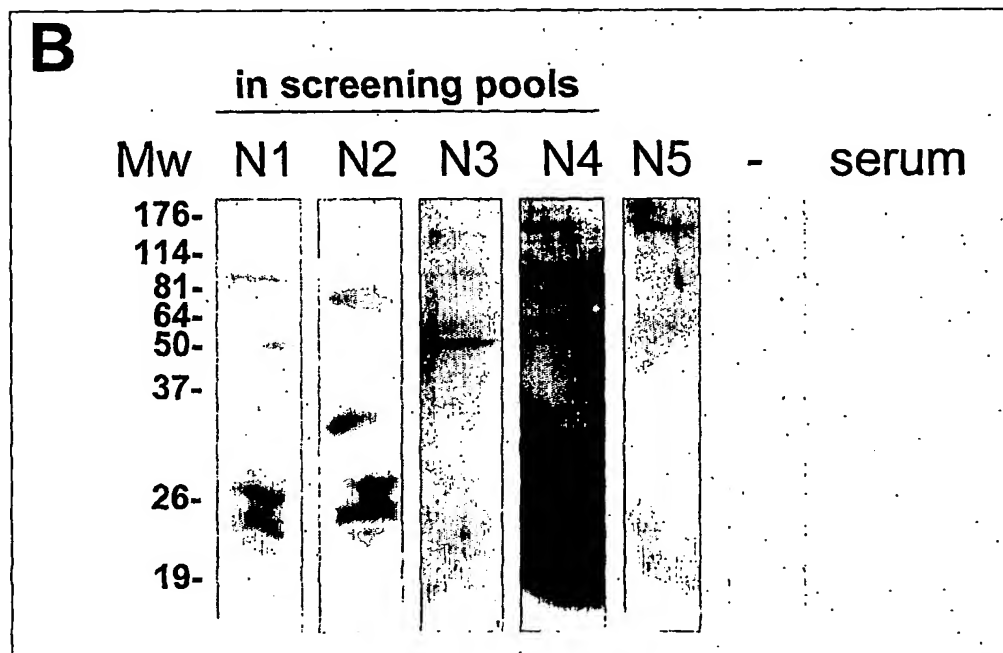
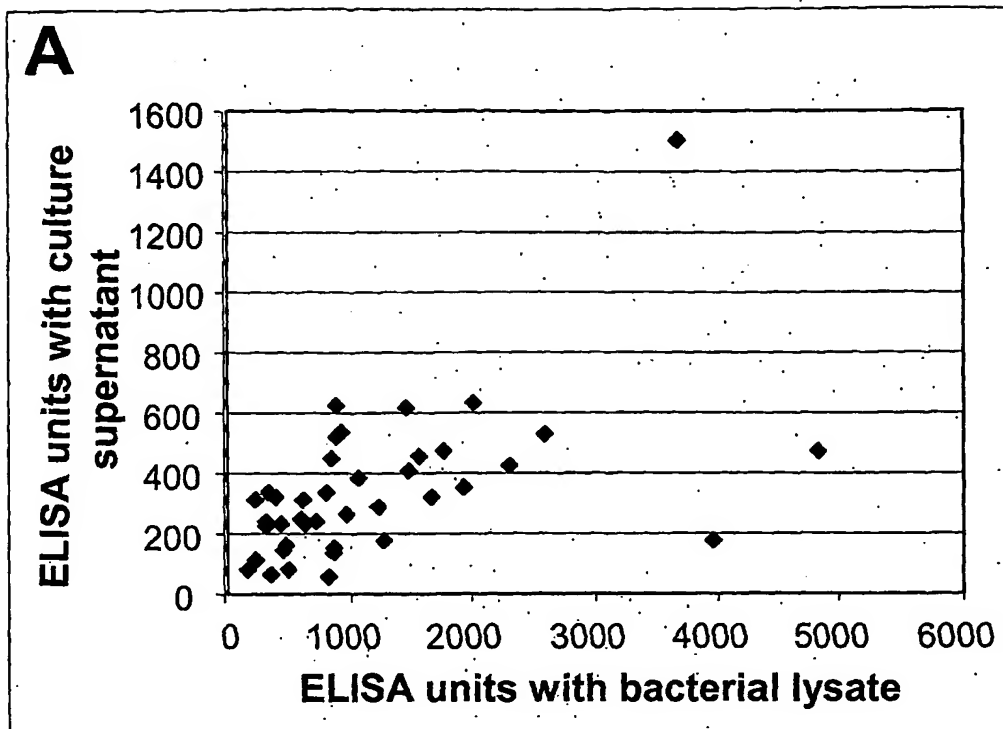


Fig. 1

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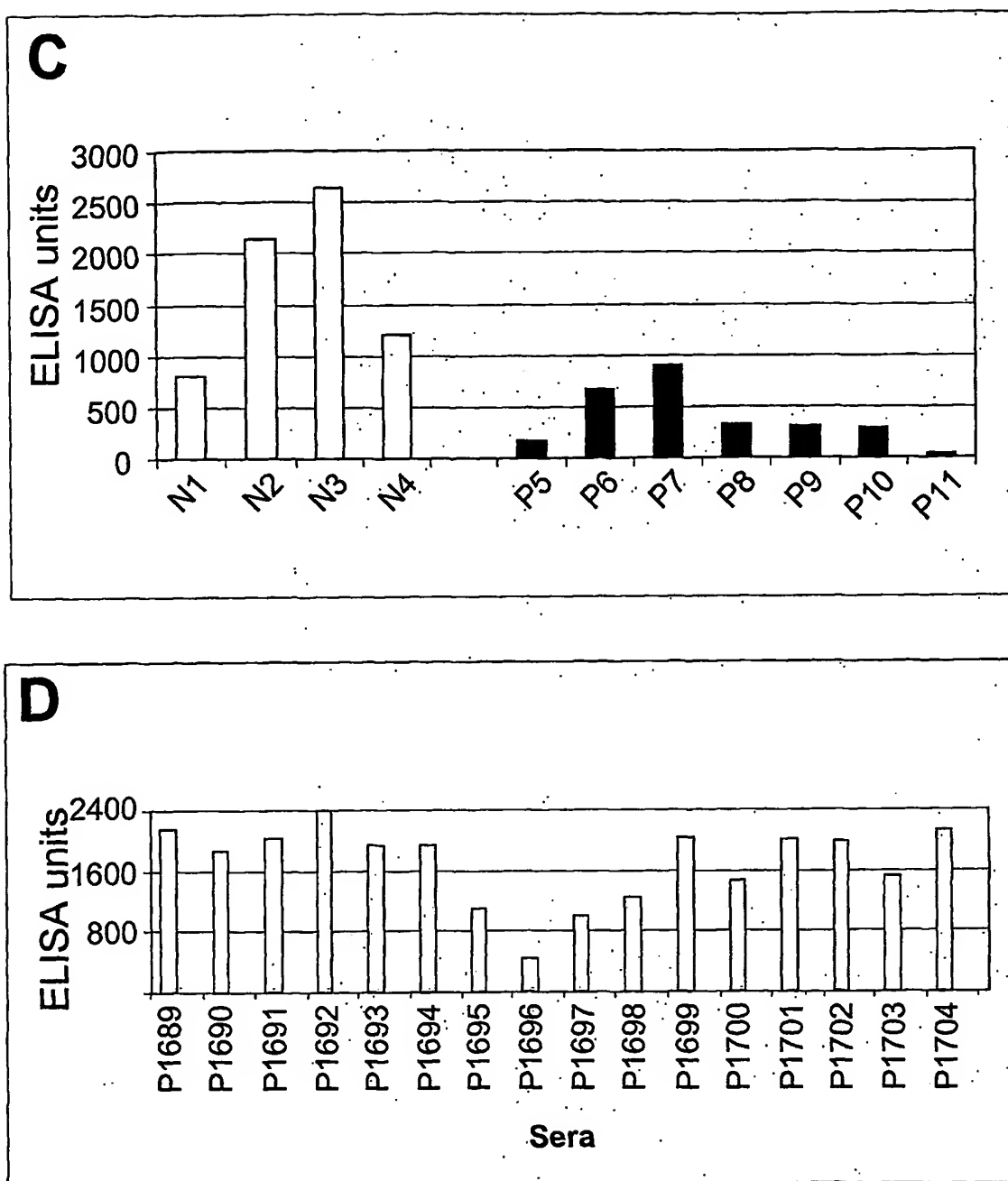


Fig. 1

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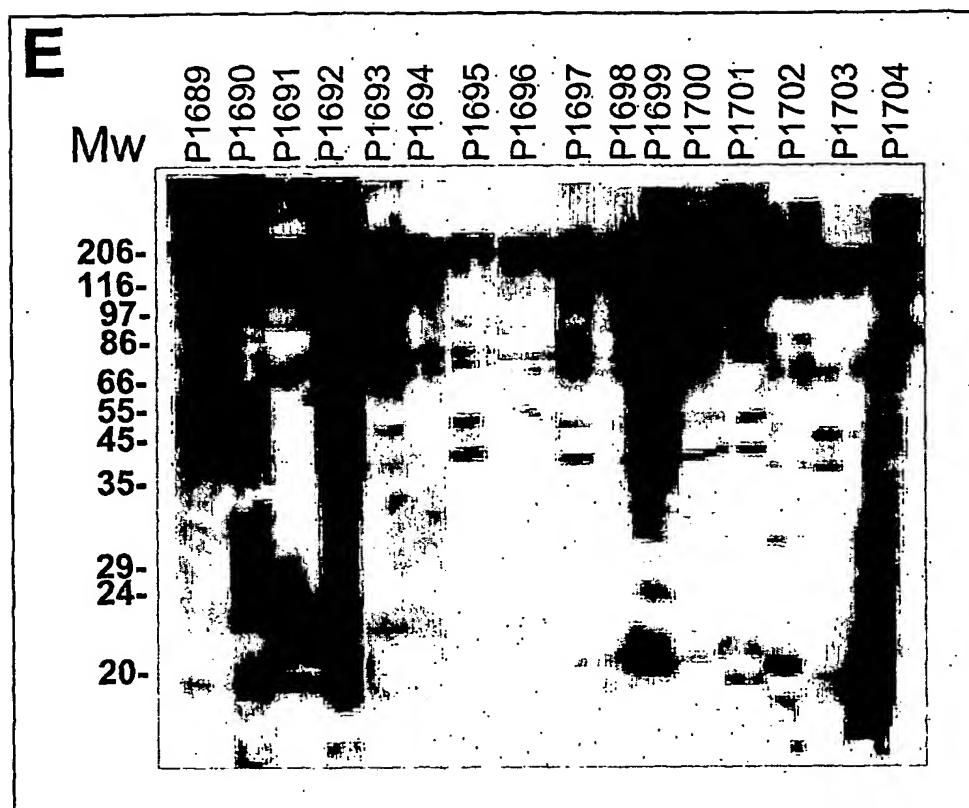


Fig. 1

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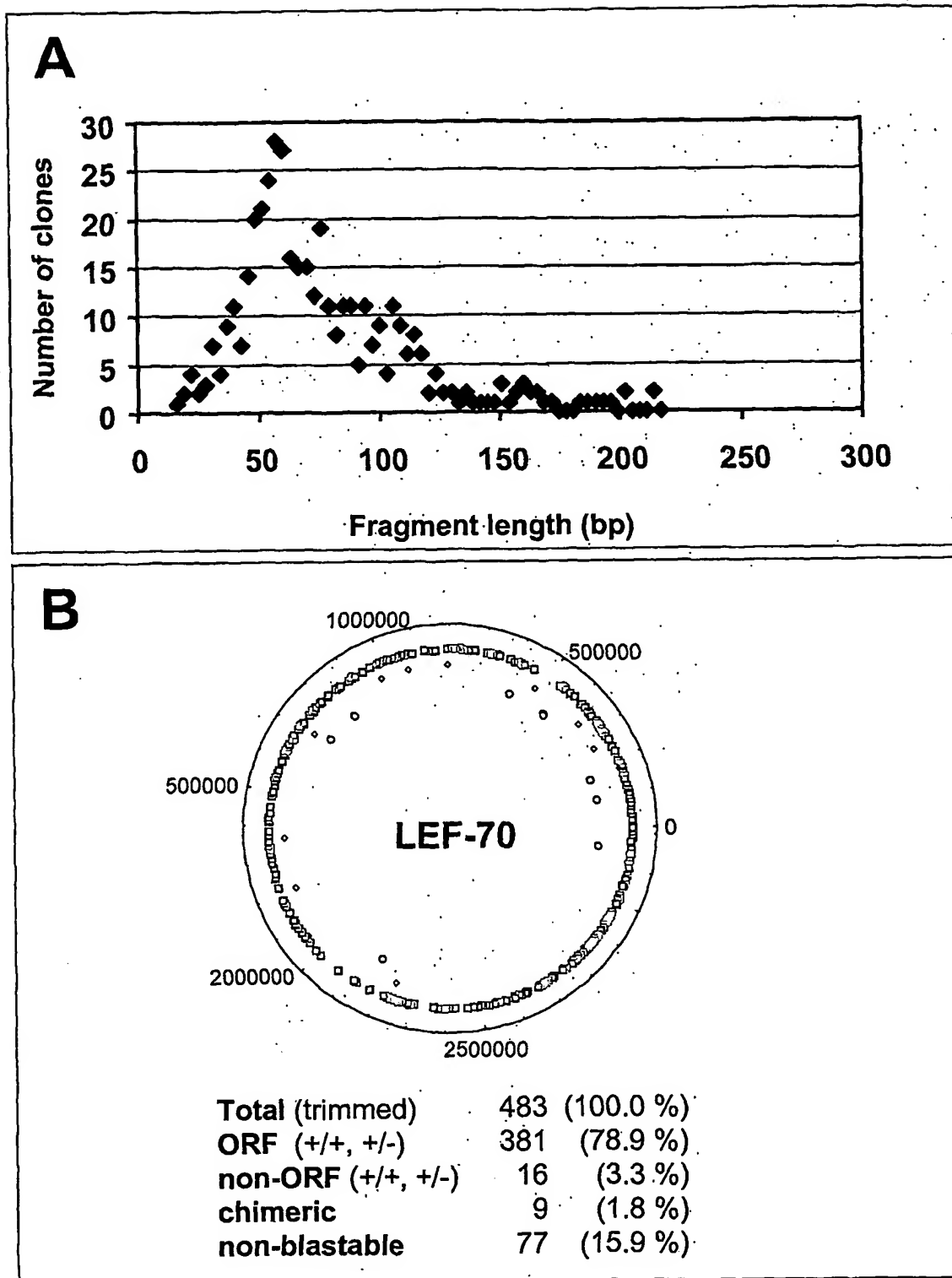


Fig. 2

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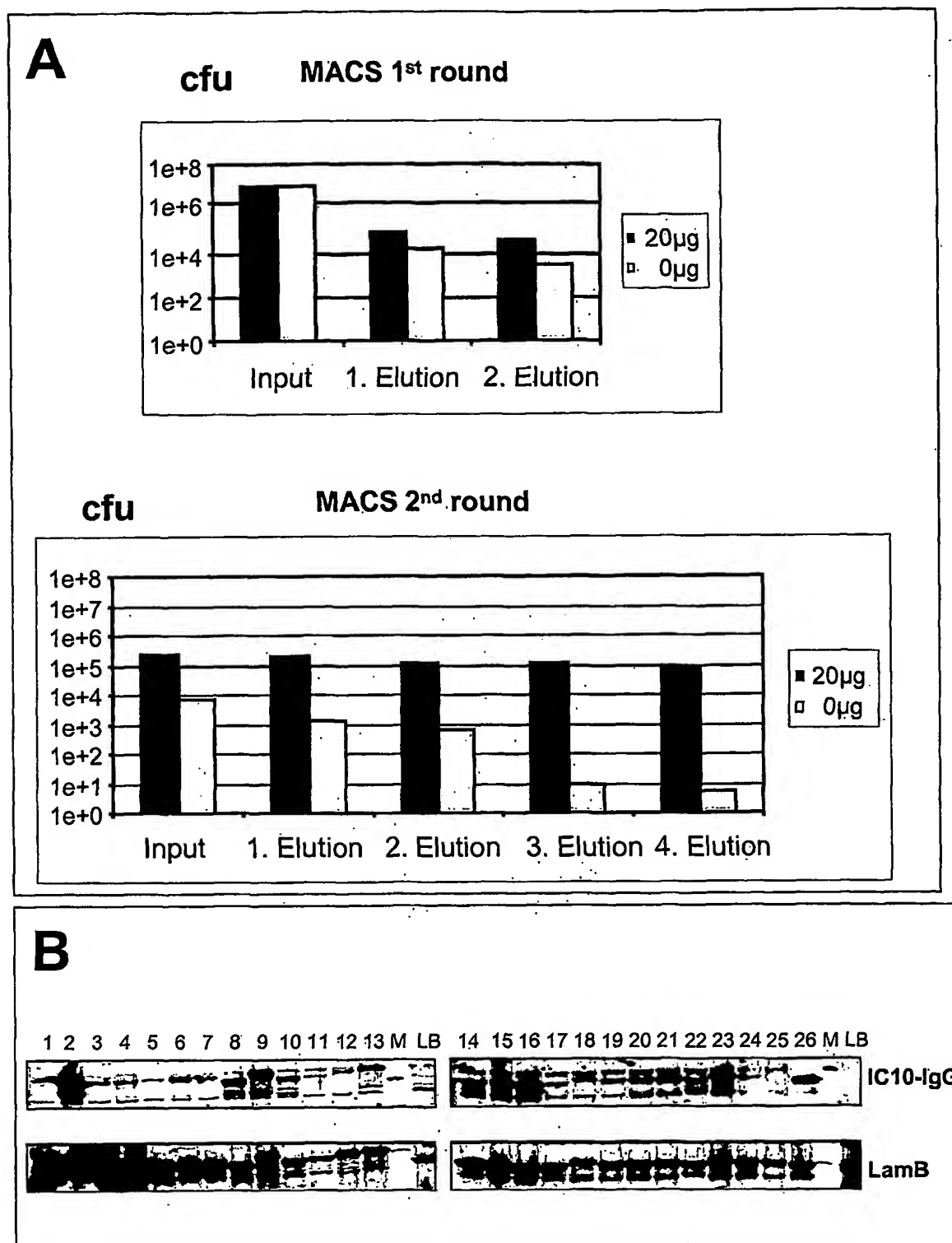


Fig. 3

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Seq ID 2

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Seq ID 11

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Seq ID 23

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Seq ID 24

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Seq ID 25

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Seq ID 26

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Seq ID 27

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Seq ID 29

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444

Seq ID 30

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Seq ID 31

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Seq ID 32

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Seq ID 33

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Seq ID 34

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Seq ID 36

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Seq ID 37

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Seq ID 42

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Seq ID 45

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Seq ID 47

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Seq ID 50

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Seq ID 51

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Seq ID 52

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Seq ID 53

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Seq ID 63

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Seq ID 65

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Seq ID 66

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Seq ID 67

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Seq ID 70

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Seq ID 71

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Seq ID 85

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Seq ID 91

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Seq ID 92

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Seq ID 93

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Seq ID 96

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Seq ID 97

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Seq ID 98

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Seq ID 154					
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Seq ID 155					
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Seq ID 156					
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Seq ID 160					
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Seq ID 161					
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Seq ID 162					
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Seq ID 163					
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Seq ID 166

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Seq ID 167

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Seq ID 168

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Seq ID 169

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Seq ID 170

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Seq ID 171

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NELANA

Seq ID 172

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Seq ID 173

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Seq ID 174

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Seq ID 175

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Seq ID 176

MSKKEINQVASSYQLYINGEWTGSGNKMIA SYNPSNGEKLAEFVDATNADVDRAVEAAQEFQTKWDVDDVTRSNLLK
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VTKEEADGLY

Seq ID 177

MGKYHELAEKIVKNVGGQENINSITHCITRLRFKLKDESQANDDVLEKNMDGVVTVMKSGGQYQVVIGNHVPAYVEEVVSI
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Seq ID 178

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QGSKAPIAQLADKISGVFVPIVIGLAVLSGLAWFFLGQESWIFALTTISVLVIAACPCALGLATPTAIMVGTGKGAENGVL
IKSGDALETHKIQTIVFDKTTGTEGKPVVTDILVADSALSEAELLTLAASAEQGSSEHPLGEAIVGAAKERQLPLAEGSD
PSAIPGHGIRVTVNERVLLGNIKLMKEEAIELSTFVQADRLAEKGKTPMFVAKDGSFAGIIAVADTVKDSQTAIARLH
KMGIEAVMITGDNKRTAARAKQVGIDRVLSSEVLPEDKALEVKKLQAEKKVAMVGDGINDAPALAAQADVGAIGSGGTDVA
MESADIVLMRSDLMVPTAVELS KATIKNIKENLFWAFAYNTLGI PVAMGVHLHLFGGPELLSEFMIAAAMSFSSVSVLLNAL
RLKGFKPSTVKRTSGSQK

Seq ID 179

MKKKILAGALVALFFMPTAMFAAKGDQGVDAIYQGEQGRFGYAHDKFAIAQIGGYNASGIYEQYTYKTQVASAIAQGKRA
HTYIWDYTWGNMDIAKTTMDYFLPRIQTPKNSIVALDFEHGALASVPDGYGGYVSSDAEKAANTETILYGMRRIKQAGYTP
MYYSYKPFPTLHVNYQQIIKEFPNSLWIAAYPIDGVS PYPLYAYFPMDGIGI WQFTSAYIAGGLDGNVLDLTGITDSGYTD
TNKPTDTPATDAGEIEIKIPNSDVKVGDTVKKFNVDAWATGEAIPQWVGNSYKQVEVTGSRVLEGLISLWSKGDIEL
LPDATVVPDQKQPEATHVVOYGETLSSIAYQYGTDTLAALNGLANPNLIYPGQVLKVNVSATSNVYTVKYGDNLSSIAAK
LGTTYQALALNGLANPNLIYPGQTLNY

Seq ID 180

LPMTIKQEQTIPKDTVETTTDLIRNLTTIKDNTGEFLDLDFGLKVDDKSWTIWNWPQGVGLYGIYKNYRNTKSEKALQVV
NDWPEGRMQBAGPPKNVNTMAPLLTMAYLYEDTKDSKYIPYLEQWAEVWMEEMPRTEGGLQHATYGPENKNQLWDDTLM
TVLPLAKIGKILNRLDYLREAKHQFLIHIKYLQDKKSGLWYHGWTFEGNHNYAEALWARGNCWITAIPIEIELELPKGD
SLREFLLSTLNAQVAAALAKYQDESGLWHTLINDSNSYLESSATAGFAYGILKAVHKKYISSEYEEVANKAIAAGLNEIDET
GEVQHSVSGTGMGMDNLDYFTIGMTAMPYQGSLLTILCLTELLVSYC

Seq ID 181

MKAKKQYKTYKAKNHWVTVPIFLSVLGAVGLATDNVQAEKLDTPETTTVPNNPDLOSEKFTPKTAVSBEATVQKDTTS
QPTKVEEVAPENKGTQSSATPNDTTNAQQPTVGAESKSAEQPVVSPETTNEPLGQPTVEVAPAEVNEVNSTSIKKEFTPD
VDKAVDVKKDPNITVVEKPAEDLGNVSSKDLAAKEKEDQLQKEQAKKIAQQAELKAKNEKIAKENABIAAKNKAERK
YEKEVAEYNKHNENSYNVNEAISKNLVPDQSVVTKDKTSSIKGKFIKATDFNKVNAGDSKDIPTKLKMDGGKVTGNFQ
NSFVKEANLGSNGGYAVLLLEKNKPVTVTTGLNASYLGKRTKAEFVYELQSSPSQSGTLNAVFSNDPIITAFITGNRVNG
KDVKTRLTIKFFDASGKEVLPDKDSPFAYALSSLSLTKNGGHAEFVSDFGANNAFKYINGSYVKKQADGKFYSPEIDY
GTGPGSLKNSDWDVAVGHKNAYFGSGVGLANGRISFSFGMTKGSNVPVSSAQWFAFSTNLNAQSVKPIPNYGNPKEPEKA
TIEFNRYKANVVPVLVPNKEVTDGQKNVNDLVNKRGSLSQYIVTGDTTKLAKVDPKTVTKQGIKRDFTDAEKVTIDLSKV
YQADASLNEKDSKAVAAAINSGRAKDVSTASYDLHLDQNTVTAMMKTNADGSIVLAMGYKYLVLVFPVKNVEGDFENTAVQ
LTNDGETVTNTVINHVPGSNPSKDVKADKNGTVGVSLSLHDKDIPLOTKIYYEVKSSERPANYGGITKEWGMNDVLDTHDR
FTGKWHAITNYDLKVGDKTLKAGTDISAYITLLENKDNKDLTFTMNQALLAALNEGSNKVGRQAWSVYLEVERIKTGDVENT
QTEYNKELMRSNTVVTHTPDDPKPTKAVHNKKGEDINHGVARGDVLSYEMTDLKGYDKDFAFDVTDLATGVSFDDYD
ETKVTPIKDLLRVKDSKGVDTINQFTISWDDAKGTVTISAKDPQAFILAHGGQELRVTLPTKVADVSGDIYNSAEQNTFG
QRIKNTVNVNHPKVNPKDVKVIVKVGDKQSQNGATIKLGEKFFYEFTSSDIPAEYAGVVEWSISDKLDVVKHDKFSGQWSV
FANSNFVLADGTVKNKGDDISKLFMTTFEQGVVKITASQAFLDAMNLKKNKNVAHSWKAFIGVERIAAGDVYNTIEESFNN
ETIKNTVVTHTPEKPKPTPEKTVIVPPTPKTPQAPVEPLVVEKASVEPELPQTGEKQNVLLTVAGSLAAMLGLAGLGFKR
RKETK

Seq ID 182

MKKILFASLFSATLLFGGSEISAFQAEIIPDDITTPPIEVPTPESTPEKPTDPTPIEPVDPVEPPIPTPEPTPEPTT
PTEPTTPTPEPSEPEQPTESKPVSEPEKPVTPSPKPAEPEKTVPTKPTSEKPVQPAEPSKPIDVVVTPTEGELNHAGNGTQQ
PTVPIETSNLAETHTVPSVTPITTTDGENIVAVEKGVPLTQTAEGLKPIQSSYKVLPSGNVEVKGDGKMKVLPYTGEM
NIFLSAVGGILSVVSGFVIFKKRKA

Seq ID 183

MGSFGLMLKQGGNDVLLIDGWQEHINAIKENGKANYNGEITVKVSVINQNEVPTGEQFDLIILFTKAMQLEKMLQDVK
PLIADHTEVLCLLNGIGHEDVIEKFVPMKIFIGNTMWTAGLEGPGKAKLFGSGSVLQNLGIGQESAKKLAETLSASGL
NAKYSNDNIHYSIYKACVNGTMMGLCTILDVNMAGLGATKPAHDMVVTIVNEFAAFAAKENVNLDIPEVIEHETCFDPTT
IGMHFPMHQDLIKNNRLTEIDYINGAISRKGGYGVVTPYCDFLTQLVHSHKEILGAK

Seq ID 184

MQTEEMQSGDGRGRRLRIFFGFAAGVGTYGMLEAHELLMMGKHVVVGYIEPHDRPDTRNLLLEGLPQIPPKNIIFYKQMV
LTEPDIDQIQKPEIIVLIDELAHSNAEGSRNRKRYQDIDELLNAGIDVFTTVNVQHIESLNDIVEEVGTIEVKETVDPDTF
LRQATIRVIDVEPELIERLEQGIYANENAKRALKNFFIPQKLDQLRGLAIQRASDHINRISGKTIGIQSKLLTVVNDAP
PKMTTEKICRWTAARLAQGLVVDWTVIQVQTQENTPTNIPLADKLGAEVISIEEDDSFETIVEFAKMTGVTDIIMGKNLRQPW
YEKIFIRAFDDRLRLKRLKDTLHLIPFNEEKRSLEFFTRKVIKGGKDLVIAIGGVFLATVVTHLMQYIHVGDQNLMLIYI
SFLVLVARTTSGYFWSLSILSVLSFNWFFVEPLYSLTVYKQGYPTLLMLLVVALMSSNLMIRLKKQADTSMKKEHQME
ILYELNKRYVVLVESRKQILDISATYLSRLLEREVIFDRQVKTESVHCINEKKSILNNEDEAAVAFWAAKNQKEAGNGTDT
LNGAKGYFLPIAAGRKTAVLGLERNADLDLENDQLNLYKLVLTLQAVILEQTELEKEQVLENEREKVRNLLRAVSH
DLRTPLTVISGIAETLIGINDLKESTQRKLLKDIQESBQWLIRMVENLLSITRINMDTMKNKTAEPVEEVIEAVYKHLRK
VYPDGQVILHPEEVIFFQADPILIEQALFNLIENAFRHGENDLPVKLNIVQKEQTVFIEIENHGEIPLKQFQKIQSNLSG
TNEVPVDSKNGLGIGLSIVKTIHVAHNGMEMTIGRGKTLVRIYLK

Seq ID 185

MKNFYKKKFPALTDQGAELTKASISSFFVYICINMVPAPIIMMLIDELVLENAKPRWLYFAVSFVTLFMYWLLDREYENLY
NSTYKESAHLRVQIADDLSNPLSYFSKHNLSDLSTQIMSDVEGIEHAMSHAIKSGGMALFFPFIISVMLLVGNVKGMLAV
ILPTLFSFVLILLSSKKSQTANTKYDTLRENSSEFQETIELOQEINSFNLSKKVQDRFLFKKMEESERHLKVELSTFSVM
ALSSIFSYSVLAVVLVGVHLLLTGEVTILYVVGYLAAIKIKDSFDSMKEAVLEIFYLAPKIQIRAMKETSIOEGSDSP
LKSFDVLVDVSYFSDNNTPILDHISFTAKQGEVTLVVGASGSKTSILKLVSRLYDYDEGCILIDGYDIKRVSPASLFSK
IAIVFQEVTLFNTSILENTRIGNSQASDEEVKKAARLANCEDFIEKLPDGYHTLVGENGSSLSGGERQRLSIARAFKNAP
ILILDEITASLDAENKRIQESLNRLIQDKTVLIISHRLKSEIKVNKIVVMDQGVVDQGTSHSELYRRSEIYKNLIKTKL
SEKFPVYKEAQR

Seq ID 186

LTLSDTLQREVTALSYSRSDVTAETLFFCKGLNFKKIYLENAVKDGLIYVSEVPYEVPAQLGIIVTDIKKAMAVLSMAF
YDYPQNKLLIGFTGTGKTTAAYFTKYILDVATQKTAALLSTMNSTLDGKTFFKSALTPESLDLYRMATAVANGMTHF
IMEVSSQAYKTNRVYKLFVDVGIFLNIPTDHIPIEHPTFDDYFYCKRQLITHSKVIVLNREADYFPLLKETAQQQKVP
VYGSQPAPEVDYFSAVSSDSLRFIVESPADALGLAGSYHLRLGDFNKGNSALASVVGASKEEQQGIATTVPGR
MESLNTNGATVYDYAHNYDSLNNLTFVREHPDGLIVLVGSTGDKAISRRKDFGRVLSLADVAVLTTDDPASEDPA
KICQEIQAHTKEMPVYTVLDRGEALAHALSSTTADDAVLAGEGADLYQKVNVDPEYAGDFALAEAFINKN

Seq ID 187

MFLGFNEMKSKGRYVLVVLVLMVLIWLIFILSGLANGLAQGNRLAVDQWQANQVLSKEANSNLNVSVLDENVKETISGG
KIAPIGQQSLAIRPADDKKAELTNVSLFGIEKESFLMKVIEGNAFTDKNQVLASETLKNQGFKIGDKLTAGKYDEQLEIV
GFISKSSYNIVPVYITSLDTRWSIKYGNPAMAKMVNGFIVRSKDNTVEKTTNKSQVLSISDFIEKLPQYSQNLTLDM
IYFLIVLAAFIIGIFIFVMTLQKTAMFVGLKVGVPSTFLAKAVMLQTAALLAVLGAIGLALTGTITVLFLPEAMPYATNGP
RMILFSVLLILSALIGGAFSIRTIKIDPLIAGG

Seq ID 188

MQIFPYSDDPNKRYHTWNYALRQOFGEKIFKVPIDGGFDCPNRDGTVAKGCTFCVSVSGSDMIVAPSDPLPLQFQKEIQL
MHQKWPTVDQYIVYFQNFNTNTHAPVDVIRHRFBQVNVNKKGVVGLSIGTRPDCLPDEVVNYLAEINERFYLWVELGLQTTFE
ETSAAINRAHDYQTYLDGVAKLRKHGIRVCTHLINGLPEGETPAMMRENVRRITQSDIQIKLHLLHMTNTKMMRDYNEG
RLQLMSKEAYSVICDQLEMIPEIVIHRLTGDAPFETIIGPMWSLKKWEVLNAIDAEMKRRNSYQGYTVISGKEVFN

Seq ID 189

MKESMSRIERRKAQQRKTPVQWKKSTTLFSSALIVSSVGTVPALLPVTAETEEQPTNAEVAQAPTETGLVETPTTET
TPGTTEQPTIDSSSTTESSTESSKETPTTPSTEQPTADSTTPVESGTTDSSVAEITFPAPSATESEAPAVTPDDEVKVP
ARVASAQTSFALSPTQSPSEFIAELARCAQPLAQANDLYASVMMQAIVESGWGASTLSKAPNYNLFQIKGSYNGQSVYMD
TWEYLNGKWLVKKEPFRKYPVSMESFQDNAHVLTTSFQAGVYVYAGAWKSNSTSYRDATAWLTGRYATDPSPYNAKLNVI
TAYNLTYQDTPSSGGNTGGGTVPNGTGGSNNGSGTNTYTVKSGDTLNKIAAQYGVSVANLRSWNGISGDLI FVGQKLI V
KGASGNTGGSGSGSGSNNGSGTNTYTVKSGDTLNKIAAQYGVSVANLRSWNGISGDLI FVGQKLI V KKGASGNTGGSGN
GSNNNGSGTNTYTVKSGDTLNKIAAQYGVSVANLRSWNGISGDLI FAGQKIIVKKGTSNTGGSGNNGSGNNNGSGTNTY
TIKSGDTLNKISAQFGVSVANLQAWNNISGSLIFAGQKIIVKKGANSSTNTNKPNNNGGATTSTYTIKSGDTLNKISAQF
GVSVANLRSWNGIKGDLI FAGQTIIVKKGASAGGNASSTNSASGRHTVKS GDSLWGLSMQYGISIQKIKQLNGLSGDTIY
IGQTLKVG

Seq ID 190

MMIVLLMSSCLFLFVSYQLYLRLLVEKLDSEDRTLKRKINFYKYQKNSRLMIYLLFSVCLLSVLLLGIVYTYTYQLNQRNI
RMEQRIERLAQGGATQGDKIKKQAIKKTALNQFPWKQAVSAESAVALTNYELQLAREWRPYLGETSITMIRSEKTQTLTSL
VFSVGLSYHEFQTGDQDNIVALIALNSVKEITMIDFNFTYRDQEQLTVKSIDTYARETLEINLEPVVIS

Seq ID 191

MSNYTIIDLNQPHLIPSAQWFSQKWQIPABAYQASMQEMLTSENHVPWHVCLNKQODIVAGVGVIDNDFHNRADLT
LCAIYVEPTFRHQLAGILLTTVGDFLANS GFKKLYLLTDHTTFYERYDWEFLTMVTTEEQSCARIYQKQLRQ

Seq ID 192

MKNSENDYIQLSQILPGLLTAFLVACLKFLAIWLPSLGAATIAILLGIFLGNTFVRGANLNRGTVKVAESKLEFVSVLL
GTTVTFTQIAQIGLQGVAFILIQMSLTIIFAYLIGKKLAFSDNMSLIMAGGNAVCGSSAIAIAPAIQADEEEKQIITLV
NLLGTVMMLTLPILSGILYGTNLLARSALIGGTLQSVGVQVVASANMVNENAVQLAMLFKIMRIVLLVAVVYLFGRFKQSKT
AESBAELVETKSSALPWYVVGFFIACVFNLSLHFPVVISETAHFFSSWFETTLAAIIGLRLDFKKFFQEGKRFLIYGLS
VGTVQVVLAILLLALLQF

Seq ID 193

MAKTGMVGLDIGTTSVKVVVAEYIEGQMNIIIGVGNKSDGLNRGIVVDIDQTVQAIQRAVRQAEKAGIQIKSVNVGLPA
NLLVESQCMIAVSSSEKETDEDVRNVASALVRSTPPEHQIVAILPQDFTVDGFEKIDPRGMLGVRMEMFGVVYTG
KTIHNRKCKVEKAGLGINELVITPLALTETILTDEKDFGTIVIDMGGGQTTTSVIHDKQLKFTHVNQEGGEFTTKDISI
VLNTSFNNAEALKINYGDAYPERTSANEEFPVDVIGKSEFVRVDERYLSEIIEARVEQILRKSKEVLDEIDAFELPGGVVL
TGAASMPGIVDLAQEIFEANVKLYVPHMGLRNPVFANVISIVEYSAQLNDIYHIAKYAIPGEKSKPAQSVAVQQ
EVRYDTYAEQPEEYEEFNERESGEKVTGKIKDFFSNIFD

Seq ID 194

MAKKTIMLVCSAGMSTSLVTKMQKAAEDRGMEADIFAVSASEADTNLENKEVNVLLLGPPQVRFMKGQFEQKLQPKGIPLD
VINMADYGMNGEKVLDAQAISLMG

Seq ID 195

MKKEIIVYTTISDSIGETSQKLLAAASAQYPDISFLNRYNFSFVTTEBELLEILKDALKDKALVVSTLVSKQLITAAKEFSE
RTGLLYLDLMAPPFELIQAKAGVDFIEEPGRRHQLDRAYFDKISAIHFVAVYDDGKNPQGFLLSDIILLGVSRSTSKTPVSM
YLANQGYRVSNLPLIPEVPLPPILEEMDPQKMIGLVCSPETLQGISSRLASLGLGNETS YTNVERIEQBLAYAEIIFAKY
GIPVIDVTAKSVEETAFLIKEKLDERN

Seq ID 196

MEWIEIKHATQNNLNISVNI PKKQLTVVTGLSGSGKSLVFDTLAESRRELNDTFSSVQNYLPKYGRPEVEKIEINLVP
AIVIDQKKVAGNSRSTVGTYTDIYTFRLLLFSRAGSPFVGYSDTFSNHPDGKCPCTCDGLGKITEINLHQLVDYDKSLNKG
PIDPFTFTVGNWRWKRYAHSGFLDCKIKDYSPEELALFLYAPQOKLANPPKEWPHALYEGIVPRMQRSLHTDEGKRH
QKYLNFHFTVKRCPDCLGSRVNERVRSCKINQKSADAVDMPLTELHSFIRSMDSLKIQTIEELLVRLBALINIGLSYLT
LGRATETLSGGEAQRIKIAKYVNSALNDIMYILDEPSAGLHPKDIERISRALLNLKNKGNTVVLVEHNPQLIREADFIIDI
GPFAGENGHGVQFSGTYDAFLVSKTLTSQALQEPPLNDQPRKARKSLSEIHAETHNLNLSVEVPLGLVLTVCVAGSGK
SSLAEIYQKAQADNQEI IHSQKSITANLRSTPMTYLNIFDKVRKLFABEENHVS PALFSYNSKGACPTCKGKGLIVSDMS
FMEDVTSICETCHGTRYKEEVLHYLYNGKNIVEVLALSVKGDYDFDKDQPPALS LKNLLEVLGLSYLKLNSLSTLSGGELQ
RVKLADTLHQKKAIFYLDEPTDGLHLIDIQOSLQLFNRMVEBGNLILLEHIDVIKSADWLIELGPEGGKNGGQLLFTGT
PANMLNSTHSITKGYL

Seq ID 197

MKLLKMLGCVGLLLALTACQAGTNSADSNKAAEQKIAISSEAAISTMEPHTAGDTTSTLVMNQVYEGLYVLGKEDLELG
VAAEPAISEDET VYTFKIREDAKWSNDDPVTANDFVYAWQVAVSPKSGSIHQALPFDVIRNAKIEALEGADVNTLGVKAL
DDKTLEITLERPTPYLKSLLSFPVLPQNEKYIKEQGDKYATDAEHLIYNGPFKLKEWDNASSDDWYTKENDTYWDAEKVK
LTEAKVSVIKSPTTAVNLFDSELDVNVNLSGSEFIPGYVDNPAFLSI PQFVYTFKMNVSVRDGKPNPALANNNIRKALAAQ
FDKESFVKEVLQDQSTADQVI PPGQTIAPDGTDFTKLAACKNNYLYTDTAKAKEFWKGGKEIGLDKI KLEFLTDDTDSA
KKAABFFQFQL

EENLDGLEVNVTQVFPFTIRVDRDQTRDYDLELSEGWGTDYRDLPTVMRIFTSDSTLGGVTFKSDTYDQLIQETRTTHAADQE
ARLNDFAQAQDILVNQETVLAPTYNRSISVLANQKIKDLYWHSFGPTYSLKWAYVN

Seq ID 198

MKQLKKVWTVSTLLLLPLFTSVLGTTFATAEENGESQAQLVIHKKKMTDLEFDPLIQNSGKEMSEFDKYQGLADVTFSIYN
VTNEFYEQRAAGASVDAKQAVQSLTPGKPVAGQTTDANGNVTVQLPKQNGKDAVYTIKEEPKEGVVAATNMVAVFPVYE
MIKQTDGSKYKGTBELAVVHIYPKNVVANDGSLKKVGTAEENGLNGAEFVISKSEGSPTVKYIQGVKGLYTWTTDKQA
KRFTGKSYBIEGENDTFAENGTEGELTVKNLEVGSSYILEEVKAPNNAELIENQTKTPTTIEANNQTPVEKTVKNDTSKVDK
TTPSLDGKDAIGEIKIKYQISVNIPLGIADKBDANKYVKFNLVDKHAALTDFDNVTSGEYAYALYDGDVVIAPENYQVTE
QANGFTVAVNPAYIPTLTGGTLKFVYFMHLNEKADPTKGFKNANVDNGHTDDQTPPTVEVVTGGKRFIKVDGDVTATQA
LAGASFVVRDQNSDTANYLKIDETTKAATWVKTKAEATPTFTTADGLVDITGLKYGTYYLEETVAPDDYVLLTNRIEFVFN
EQSYGTTENLVSPKVPNKHKGTLPTSTGGKGIYVYLGSAGVLLLLIAGVYFARRRKENA

Seq ID 199

MKTPTFGKREETLTYQTRYAAYIIVSKPENNTMVLVQAPNGAYFLPGGEIEGTETKKEATHREVLEELGISVEIGCYLGEA
DEYFYSNHRQTAYYNPGYFYVANTWRQLSEPLERTNTLHWVAPEEAVRLLKRGSHRWAVEKWLAAAS

Seq ID 200

MARVESFELDHNTVKAFYVRLAGTRONGDALVEKYDLRFLQPNKDALPTGALHTLEHLLAVNMRDELKGIIDISPMGCRGT
FYMINWDQHSPPQERDALVNVLNKVINTEVVPASAKECGNYKDHSFAAKEYAKIVLDQGISLDPFERIL

Seq ID 201

MNIPAVRLKEALTAKNIKPSDLAKKTGIGKSSISDWLAGRYEAKQDKVYRIADALDINEAWLMGQEVPMKONASTIDRIYK
KLEPQRQAIVYQFAEQQLHEQQTQAEILSFPRRDEMTELAHAGDPEKIFSKKEIEKIHVDYLDDEIDAKYQSSISSDKKED

Seq ID 202

MYKFVDNTQATHSTPLPSEALNFNGQFLEKVIPIGYQTLSSVSGRELVPSEIESYQLGIRDGKRHVYARIPERELTVKYRLSA
VNNEAFRDAENHLNVALPTEKDVSIWFNDEPEMLWFGSKSSVSDVPEGVNQVTGTFTLLLSDPYKYTRSDATSVMWGSPIT
TFQANYLMGNTGSGAFDFFILIEGGAYWGSTMITFQNRAYTMGDLOGEVRPIEIIYPTVEGLKVKPTIILTGTGRGVWIKTR
NDTINLGDFFDRSEI I IDTENFYLTNGAPMIRPMNDFYLYPNEPLYQAKDSDFRLTIRYFNRFV

Seq ID 203

MDFFSSSEIIVASLGDKAENMGKLSFVRLCILGIMAGFCIALGYLAFIRITGTAPTEWGSFNNFLGGALFPVGLIALTFVGG
ELATGNMMVMTLGLVLQKKVRVGALCYNWIVVLLTNCLOGFLVAYLFGHIVGLTEGAFLDKTVAVAQAIADPPLVAFVSGI
GCNIPVCLAVYLGALAKSYLGKMPGLWFPVMVFVVCQPHVVAFAFIIPAAIFSQSTTISWWDYLNQTLWVFLGNVAGGSL
FMAVPLIFMTKPATVKPRVEKTIQTEELYGN

Seq ID 204

MNQRLSTLGFDDKVKQQLLQFIVTAQGTNEVSELLPIADENKIQSWLNETQDGLKVQRLRGGIPIPKLENIQPHMKRIEIG
ADLNGIELAQVGRVLSTTSELTRFFDELSENEVDFERLYMWREOLEVLPELNRQLKQAIDDDGYVTDEASPALKAIRQNI
RSEQTFIREELDSIIRGNARYLSDALVTMRNERYVIVKQYKNIPIFGGVVHDQASAGQTLFIEPKQILEMNNRLRQQQIAE
RNEITRILAEBSAELVPYRREITHNAYVIGKLDIFINAKARLGKELKAVVPEISQANHVVFQARHPILNPEKAVANDIVIG
EEYQAIIVITGENTGGKTIITLKTLLQMLGQAGLPIPVEEBSKMGIFTEVFAIDIGDEQSIEQSLSTPSSMTNIVSVLKKV
DHQSLVLFDELGAGTDPQEGAAALATAILDSLGAAGAYVMATTHYPELKVYGYNRAGTINASMEFVDVTLSPYRLLIGVPG
RSNAFEISKRLGLDONSIIBAKQINDGESQDLNEMI EDLENRRKMAETEYLEARHYVDESAAHLKELKEAYQVFFEREKE
LQKARKEANKILAEAEENAEITISDIRKMQLSESGQGGVKEHQLIDAKTQLSQLHHEETKLAKNKVLKKAKEQKKLKAGDE
VIVNTYQGRITLLKNDGKGQWQVQLGILKMNVSIEDMTFVAPQKEAKPRVTVRSAESSHVGTQLDLRGKRYEALAEVDQ
YIDAAILAGYPQVTVHKGCTGALRTGITFLKNERSVKSYEFAPQNGGNGATVVKFQ

Seq ID 205

MKEITGATRLAGLFAKPSQHSISPLIHNTAFQNLGVDARYLAFDVGQETLPQAIETRTFHLGANLSMPNKVAAVSYMDE
LSPTAQLVGAINTVNKGDKLYGDSTDGTGFMWSLKEKKVDVPQNKMTILGTGGAALSIIAQALDGVKBIAYVNRKSAGF
NDSQKLANFTERTNCVHNLNDLADTEKLAKDVAESVLLVNATSVGMHPAHSSPIENYAMIQPKLFVYDAIYNPRETQLL
KEARLRGAETSNGLDMLLYQCAAAFEQWTGQKMPVSVVKRIENR

Seq ID 206

MTKGYVKSVELIGQSPVVKLKRMVPEGAADVFKLEFFNPGGSVKDRIALSMIQQAEDGRKPGQTIIEPTSGNTGIGL
AMVGAAALGYPVKIVMPDTMSIERRKLMQAYGADLLTPGAGMKGAIAKATALAKEHGYFMPLQFNNPANPMVHEQKTGKE
IVDVFGKRGDLDAFVSGVGTGGTVTVGVGHELKRIIPDIEIVAVEPTESPVLEGGEPGPHKIQGIGAGFVPEVLDTTVYQKVA
AVSSKDALETARLMGPKEGILVGISAGAAIKAAIDLAVELGAGKRVLALVPDNGERYLSTALYEFPE

Seq ID 207

VKNQHLTTSQGSFVGDNQNLSLTAGEFGPVLIQDVHLEKLAHFNRRERVPERVHAKGAGAHGIFKVSQSMAYTKADFLSE
VGKETPLFARFSTVAGELSSDTLRDPGRGALKFYTDEGNVDLVGNNTPIFFIRDAIKFPDFIHSQKRNPRTHLSKFEAVW
DFWSESPESLHQVTTLMSDRGIPLSFRHMHGFGSHTFKWVNAAGEVFFVKYHFKTNQGIKNLESQLABEILAGKNPDFHIED
LHNAIENQEFPSWTLVSQIIPYADALTMKETLFDVTKTVSQQEYPLIEVGTMTLNRNPNENYFAEVEQVTFSPGNFVPGIEA
SPDKLLQGRFLPAYGDHRHRVGVANSHQLPINQAKAPVNNYQDKGNMRFNNGNSEINYEFPNSYTETPKEDPTAKISSFEVEG
NVGNYSYNQDHTQANALYNLLPSEKKNLNNIAASLGQVKNQEIARQIDLFTRVNPYEGARVAQAIKQQA

Seq ID 208

Seq ID 208
MAENVQQAADIIQLIGMDNIIISVTHCQTRLRLFKIDHEQVDGKQLEKIDLYKGVFYNGQYQVILGTGIVTKVYDEIEKL
GINVVSQAEQTAILKNNETGMRKTRMRLISBIFIPIVPLVIAATGLFLGLKGVIINFDFLFLQFGASVANIPESPFQIVSVTID
TVFAFLPALIVYVSKFAFNATPVIGIVIGLMMVSPILPNAYAVATPDSGVKAIMAFGFIPIVVGQAQSVLSAJAAGIIGAKI
ELFFRRKKNMNLDDQIFTFPMTMLITFLINILGIGPILBTELVGMVDVQVWLIGLPLGLGFGVIGASPLMVILIGIHTLTM
VETSLANTGFNALITICAMYGFANVGSCLAFAKKAQDSKVKSTAIGSMLSQLFGVSEVIFGLLIRWNLKPILLCVLFTSG
LGGAILAIFPHIQSNYSGLAVIPSPMLITYSAHQVLITYLLVALLSVQCYALTSFAIPQEVLSIDKVIIRREEREVEFMQEN
TLDEQLEFSPVTGAYMNLTAVNDPVFSSEMMGKGLAIMPTNANKVYAPADGLLNLVAETGHAYGIQTDAGAEVLIHGIDTVT
LGQEVQPTQVTOGHRVKKGDLLGTDFDRKAIKEAGLDSTVMVITINTSSYLSVEPMMSDNEBITPEQIILNLNTPN

Seq ID 209

Seq ID: 203
VELVDKLRSLIQETRNLRKAKEAQQVLATFSQKQIDAIVQAVSEATFNQREKLAKMANEETGFGIYEDKRIKNAFASKVV
YDEMKDKATVGVVHDDAAKVTELVAPVGVLAGLIPSTNPTSTVIYKALISLKAANSIVFSPHPNALKSIIETVEIIQKAA
TAAGAPEGCVSVIKPTMQATSELMKNKETNLILATGNAVMKVAKSSGTPAIGVPGNGPATIERSANVPYHVAVKQIMSD
TDTNGTICASEQSIIVETVNRRAVKEELIKQGAFFLSPAEADKLAKFILRPNGTMPNPQIVGRSVQHIASLVGLSIPKDRRL
IVAEETHVGLKYPFSREKLAPIIAFYTVENWEAACALSIIEILKGEAGHTMGIIHTENKEVIREFGLRKPVSRLLVNTSGTL
GGIGASTNLVPALTLCGCGAVGGSSSTDNIGVENLFLNLRVAVGVRLDLEIRIQEGFGTSTTSVATSCETTNPQEBLVAVQ
VLARLN

Seq ID 210

Seq ID 210
MNELNKTPKBEIVKVELDQYIVGQQAAKKSVAVALFNRYRRLQLBENMQODITPKNLLMIGPTGVGKTEIARRLAKIVNAPFV
VKEATKIVTEGVYVGRDVESMVRDLVENAIQIVEKQQYSRVYAQALKKANQRLVKVLVPGIKKEQKQAGGNQFQGMNMFNM
AQQQQEAQEVEYTEDIRTNRRITILEQLEKGLLDNREVITIEIEPKKTMTPAMNNGLEQMGIDLNETLGALSPKKKIERTVTVK
RAQELIVKEESAHSKLVNDADIHSRAIRLABSSGIIFIDEIDKITSKSQNSGEVSRGEQVORDILPIVBSQVNTKYGPLQTD
HILFIASGAFHILSKSPDLIPELQGFPRIVEELDDLTAADFVSILTEPNNALIKQVVALIGTEGVRSVFTKEKRELAHIAY
DVNRDITNIGARRLTILIERLLEDLLYEAPDMQMGETITITEAYVNEKLNIDIVQNEIDLRYIL

Seq ID 211

MAKRIIIMFDIESKSYQAFSEIKMQAERQL
KGEQMAVVTHVNDGQHOFKINDFIDFTGNNHTSKDSMIGMLVGILGGPLGILFGWFAQSMYGASKDAKEIQRAQTVFEHVI
QKIDEGCQTGLLLIAEEDNRPLNQLVMFDLGGBITRLDLFEVQQEINDANEVANEAKQSQWAKKBEQHKEATSKEE

Seq ID 212

Seq ID# 212
MEKQITIIDVAREANVSMATVSRVVNGNPVKPATREKKVLEVIDRLDYPNNAVARGLASKKTTTVGVIIPDVSNAFFASL
ARGIDDVATMYKYNILANSDDGDDQKEVTVLNNLLAKQVDGIIIFMGHRIITDIRGEFSRSKTPVVLAGSIDPBEQVGSVNI
DYTEATKDATAATLAKNGNKKIAFVSGALIDPINGQNRMKGYKEALAENGLSYNEGLVFSEYKFKAGINLARVRNMSGATA
AFVTDDELAIGLLDGMLDAGVKVPPEEFIBITSNNSLLTEVSRPRLSSITQPLYDIGAVSMRLLTKLNNKEEIEKTVVLPY
GIDQKGSTK

Seq ID 213

Seq ID 213
LKKYNDWNVVVRFLKVMALIVGYLLITNYQHVFVHSVSGILGILSPFITGTVIAYLLSGSQKKIEGLLERVPLFVVKKAK
HGLSVLLLYLIILFIVFLITLNYIVPLLISNLVDLANSPLTFYDHMVQFVMSLEDKGILKTAATIEKYLNSVLKDLSPERFLN
QWTQALFSLGTLTKNVSSFFLNAPLTLIIISYVALVFKQSILTFVGRKAHKLSEKVKYQTQTWLNTTNKIFYKFI SCQFLD
ACTIGVSSSTILLSILNVKFAVTGLGILGICNMIPYFGSIFASIVAGKVTITLFGGVQTAITVLLVLLILQQIDGNIIGPRIM
ADAGNVNPLIIIVLITIGAGYFVGLMFLAVPAVAIIKIIVSEKWLNESKENDKIVDSIES

Seq ID 214

Seq ID 214
MKKFSIRKISAGFLFLILVTLIAGFSLSANAEFYIVPAESHSRQKRSLDLPEDRRQEVADTTEAPFASIGRIISPASKPGY
ISLGTGFVVGTTNTIVTNNEVAESFKNKAKVLNPNNAKDPAWFYPGRDGSATPPGKFKVIDVAFSPNADLAVVTVGKQNDPRPDG
PELGLITPFLVFLKKFESSDTHVTISGYPGEKHNTQWSHENDLFTSNFTDLNPLLFYDIDTTGGQSGSPIYNDQVEVVG
VH
SNGGIKOTGNHGQRLNEVNYNFTVNRVNEEENKRLSAPVAA

Seq ID 215

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Seq ID 215
MKRKIKNQILLVLVLSILVILLSLEVAPVVTLAAELPSSSLQVALSSETTITSEEKVTETTETTATSTSTSTSSSSSSSSSSST
DTTBTBSTSQSTTETTTTTNTSSSETKKEPTEPTVSSEITQFVEQSQQPQPVVTKQEPBEPQVPEANNNFVNEQAVSLNPSL
KVDEIASSNLKGYELPLLSSFGKEKRAVVVAEALRHVGKTKKEFNLTQEQALTSSFLAQIKIYQQLFKDIGSTPQEQMTFGK
VRSIKEAPEGDLIFWQTAGBKTLQGVNYVLGKTYLAAAESKDEKPEVIAQLENIATQPPDSKEEKLRVLVNPFKFEI
LTSIKEVLVATLQSGAFMQKSELTQNTAKIKIGETARELGEKYDVFAEVLQAALLESQSGESQLAKEPYYNLFGVKGSFQG
NSVSFSTKEADQRGQLYTISAGFRDYGGYNDSLQDYVQLLRQSIDGNQDFYKPAWRSEARNYLQATRFLTGKATDKQYDN
KLNSLAVYNLTQFDLPKTVDLIIIQSKNKLSEAEQQQMHPVYDGINYNRSGSYVPGQCTWYVYNRFKQLGTSVDDEFMGN
GSDWGEKGRALGYQVSSLPKAGRAISFQPGVAGADNQYGHVAFPEAVTSDGIIISESNVINDQTISYRVLPNVLIAYSSGVT
YIGA
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Seq ID 216

Seq ID 216
VDASAYLGPDDAAHSFGLRKTPLRLQAMYDKPGTIYLYTMHTHLILNMVTQEQGGKPGQVMIRAIPEVGEVDKMIENRQGRQ
GVELTNGPGKLVAAALGIDQLYQGSIFSSSLRLVPEKRRKFPKKIEALPRIGIPNKGRTTELPLRYVVAGNPYISKQKRTAV
DQIDFGWKDEENEKSNNNAHLRGTT

Seq ID 217

Seq ID 217
MAKKKNVSVISVEKPTWFLPLTDETGAFPVYGAPITIGTAVSIKPDVITETTPDYGDSVVDQYVAFGGAEVTTLETNGYQNE
VLAETITGGKKLKGGLVLRSDDIASDGAFAFYRRRKSNKGKYRTIFYKGFALTSDBTSTLEGSSVSYTHPEWTGSFVDVPGL

MFLGLIIFLPGVFLGRLGLGLTLLANCLRLVVGNTFFFAAILLILYGLLVMTYKDFPLKGRGPIFGAVLIYISVLLFFH
AFMFRNVSGSQPDILGNTWEFLQSDLKANQVTONVGGGMIGALLYQSTYFLVAQFSGYLATLTLLAGIFLMSMWDFQOIV
DHFQSIQDRLSHVQSAQSPARQEEKAKRAAKKAERQAKIEAAAOQKIQERERMEQAAAEKRLTKTFVETHQPMVEEPA
APTVPQIDSFQQNQAMFPLAATKPKQREKEEADEAGVLEFISEEAEDRDYQLPFLDLDTIQATDSGSEYKEIEKN
IGVLGTEFKFSFGVDAKVKASLPGSVTFKEVQPAVGKVSCLVNLTDIALALAKDVRMBAPTIPGKSLIGIEVNSAIST
VSFRDIVEAQPSHPDKLLEVPLGRDISGMVQTADLSKMPHLLIAGSTGSGKSAVINGIITGILMQAKPHEVKLMMIDPKMV
ELNVYNGIPELLTVPVVTNPKAAQALQKVLVQEMEFRYEKFAATGVRNIITGYNQLIQKNAEDGENRPILPFIIVIVDELAD
LMMVASNEVEDAIIRLAQMARAAQHMLITAVSPVDVTGITKIANVPSRMAFVSSGTDSTRTIIDTNGAEKLLGRGDMLF
LPMGENKPIRIQGAFISDQSEVERVAVFVTDQBEAYQSESMMPDTEPTSGGGEAPQDELFEAKNLVVMQTAISILLQRR
FRIGYNRAARLVDLEAHGVIGPPSEGSKPRKVFLQAESEEAATETPEQ

MRKSFNLAVQALTVQYQGRITALNNHVTIPSGKITGIIGPNGAGKSTFIKGLLGLIKTKERDVLNNQQAIDQQKTTIAIYVE
QRSALDLSFPISVFETVLLGTYPNLGLLRPGKKEKQAAMAALKMVQLEDYAQRQIGELSGGQLQRVFIARVLAQGAEIFI
LDEPFVQIDMSSEKVIDILKSLKNQGKMIIVVHDLHKVSYGFDELIVLKNRLIAAGPVEQTFTAETLQEAYGDLLGDLL
IQGVAK

MIGLELFTTVPQADEVIRSIVETYHDEKDIVIGAGTVLDAVTLARLAIMAGAEFIVSPSFNRETAEI CNLYQVPYLPGCCMTI
 TEMQTALKSGVDIVKLFPGSTYGPSIIISAFQAPLPYLNIMPTGGVSLDNMASWFEAGVTAVGVGGNLLAHAKTGEFGKVTA
 LAKOYMAKFRIKEN

LAGVTKEQIAKAKENDLLSYLQAYEPQELKSGPREYCTRTHDSLKISNGKWCNWSRGIGGRTALDYLIKVRGMDVFVGAVE
TLCGYCAPPPKAPPIKKEKQPKFFRLQPOASRCASAVVGLQDRGIDPELLGVLCMEAGILYESSCKYONCVFVRDRTAGNARA
ASLRGTRDGRFMDVEGSDKRYSFCLPAGKADCPRLAVALSPIDALSATLVLKSGGEPRDSHYLSGGTGPRALIQFLHDH
PHVTQVSLCLDNDKAGLEGMERLTQRIQNDASLRRVLVYPNPKQKGDYNEFLCTHVKAVRTAQRQDRAAR

VRIKEGRTVGLFTKVSPEEKAAIDRKMELGTSNLRAYLRKMAVDGYIVQLDMGAVLELVKLLRSVSSNVNQIARRCNSTH
 NLYEODVEDLROGYSEIWOGYNDLKKFEAL

MANKLQTVSQLADQTAKAVTRNAEGWKSYLTTASRLYKYSFDEQLLIYAQRPEDDATACASMLWNEDMRRVVKAGSKGIALI
KDGIRPRLPTVYFVDVADTRPVGRGKMPVLEWMDKHGHAAMVDLTARRYGTEKDKIGSALMEQAKHAVEEYVREHLSDLVKA
DAQDSLLEGLDDFNLEVRFNRLLTASVQYTLTLSRCLNPADYLEDDEPDGJHEFSTPAVLEHLGSAASESVMMMLLEVKKA
IRQAEKEKAQNROKIKPNPEKPLAKEPVIGYTKVKEQFNTLKRSEERSIQNGRTGIQEDGRLPDSRLDGRGGRDGGNAA
GQVQRQAADLSSGPTQGDILHLDAADRAAGTPTAGDRPAGAGTRPDRRGIIKETERKRGDDESPRFDGMAGSGQVPSRPGGG
NRTERDRLQVNQENQGEAAGEQSASVASEKPAFTQSFLLPTFTEVQOISTIAQAQTEQELRPAVSSGKVTDVIGRALTSGG
NEPDSILRIIVAFQKDPTEQSAADFLQKEYVSGSGKLGKLAGHESLWFSHSGIHIAPRGSAGGSLVTVWKQAAQIRQLLN
SQQFASQDKIDAADNEFRELSSEKLWFLRLNPSFEAKEKGLLPTIDALYGVGPPDSTA KIAALLDKPAERAKIAQEVQRFAI
SHQENPNLLRFRQRPAPSEFLTRLTDMDKPVTAPHGVEGAPARGAFVTEDEITRMFTGGSGVSEGGKFRYIAYFMQGHDAK
ECIEFLNKEYGIGGGHYIGYDEWDHGDGKILSRADDPSGNGYDPTVLNWKQVQKRJAGLIKTDTRYLNRRREKAYLPEYEFKMG
LARSLYTFQYYDPNDASKTIPHWDVDAAKKVFRPLDDPEQCAAHYKEMVKALAMVSPDERAYSLMEPTLQKMGAYLRGE
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PEPEPELVSQAFTGASETAVPJASKPEPPQDSTFNIPEGAAPIMAESPADRYDLGYGHMGNGLTVWNRLSEEHGDKYKTVAHIA
PDRTPVTPYDADNEMPIRETKIQVAAATEMSISATQDTPVFTSPQPEPERVQPGNSEPEPEKQVDAPAAVNPETPTGDSNIV
PSPAQAQAEPTGTGSPQTQEAEPKTAADGLNLTPNVEEYILNKAQYEDKLGVVRVGKTMFLFYGTDAKEAAPALGTDTKNTITR
DIPDLGMVSTITGANGQVSLKLLHEHGSVVLARPDPVEEGDAPYEIISKERSAADYIPVGMELTIDGRMLKIDSVDYNAGT
VBLQDMELRGWYPIFSESIFIPFVRQFVEEVQGEHFTAEPMQEBPERPETSOLDTAKOLIEQFAYASEYSGDDVDPSLEHTGII
AYGTTEDDGGLEVVQVDNLLDFSISQSDVGKCVETROQYGSRLRELIDMELAPLDYDMLVSVSPDIBERLKBALNQRIRWSEMA
GAREGVTEPELPIPEKQDSPEQAEAPLEVIDGGQITPTPAQRQTRRAQEBSVDSRVPFSEIILQPLRELPERHNFRTID
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TVLNAHYTSPTVIQAIYNAEQMDFTFGTVLEPSPMGIGNFFGMLPEKLAAAKLYGVELDDLTGRIARQLYQKADITVDGFE
RTDHPDDFFDLAVGNVFFGSYQVHDKRYDRQNLMIHDYFITTKLDKVRPGGIVAFITTKGMDKKNKAREALAQKADLLG
AVRLPSNAFKLANAGTEHTDILFQKRDRIPEKLPEWVEVGQTEGDQIPLNRYLTDHPHEVLGTMTMGRSMYGNETETACQF
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IGIRDSARRLIDLQLAGADDTIEIOSEQAKLNRLYDAFSAKYGLLNSTGNKLAFEQDSSYPLLCSLEIINBEGKLERKADM
TREYIQTBRVATSVDTVAEALAVSGEKAACVDLGYMASLMGGEKIPQIPEVDLGSWIEIFKNPATFPLEDDGGAHQHWQGT
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QYSPTTGSEWNVQGSKTNRDNRVVRVATYGTKRINAYEIFBQTLNQRDVRIFDKTEVDGKEVRVLNEKQTAIAQQQKQAMCE
TFKDWIFKQDPERRETLCRRYNEKFNCIIRPREYDGSHIRFAGMNPESILRTHQENAVARMLYGKNSLLAHCVGAGKTFFEMIA
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ERQAKVIRKGQIDETVTALEAKAEDGERYTIKQMEKTKNLEARKLQKLAGDKKDSVVTFEELGVDRLFPDEAHGFKNLPL
HTKMRNVAGIAQTDAQRSSDMFAKCRYMDEITGGRGIVFATGTVPVSNMVELYTMXRLQPDFTLQNGHRHFDAAWADFGB
KYVTAMELKPESGGSFRSKTRFAKLYNLPILSDISEKAAADQIADMLNLPVKAEEIITVTEPSGQVQEMVELGERASEVRG
GOVDPHLDNMLRITSDGRKALDORLONPLLEDPSDKNACVKNIVOEWAQTEIIGTOLVFCBLSTPGKDGSPNVYDD

KEKLIAGIPEAEIAFIHDANTETQKARLFAKVRRGQVRVLIGSTARMAGTINVRNVASHDLDCPWRPADLEQRAGRS
RQGNMNASVKMFYVTKGTDFDAYNWGLVENKQKFIQIMTSKSPARSAEDVDATLSYAEVKALATGDDRIREKMDLDVQV
SKLKMLKANHTSQQYEMQDKALKYYPQKIAETKLLIBALTADLPTVQAHVVKDDAFSMTVMGQTYTERKAAGBAVIAKACL
MDDPEKTVDLGEFRGPFQMLRCDGSKFRVMTKQNLTYSAELSDDAVGNVTRINNALESITERLDAQARLATLESELENAK
EADRPFFKEREELREKSARLNQLNRELRSRDPKKEDEQVEESPDLEADAGKAPVVPMPAVAAGYKPSIRQAIRNYEPPTFV
HSGMERTPRKRAVL

Seq ID 224

MPVLTSGQQRKSKQRKIRNSEYDDMEGTFDRLYAESKDKTFNHLMEIIESEENIKLAYRTIKKNTGSDTSGVDKRTIAD
LAKLSEEEYVRLIRKQFSNYHGPVRRVEIPKPNKTRPLGIPTIVDRIVQCCILQVMEPICEAKFSSENSNGFRPNRSAAET
AIAQCMRLIQVQHLHYVVDLDIKGFFDNISHTKLIHQINALGIRDKLLCIIKEMLKAPVVLNPKGKTYPARGTPOGGILS
PLLANIVLNELDWWIASQWERMPTTKTKFTRSNAGQTEIKSHAYRALRRSRLEKMHAVRYADDFKIFCATHBDVAVRYKAT
BLWLKDLRLGLEISPDKSKVNLKRQYSDFLGPKLVKVKKGKYYVRSEMSDKAYKKAHEKVSEEVKKLAYSSDDNAQFMQL
QKYNVAVAGLHEYYCISTEVSHDFSRLAFSINKQLNRNLKEDLSKKGQLRNGFIKEKYGASRQMRFLHGRFPVVLGYVQSK
NAQHKRSINKYTVKGRQIHKNLAIIDTATMLWLMRNPVKGRITIEYADNRISLYAAQYKCAVTGIPMNSHDICHCHKVPV
SNGGSDBYANLILVSKAVHILIHASSEPTIEKYLKSLNLDNKKQIEKLNKLRSMAMPPIIL

Seq ID 225

VTEKARDLFDKLMTOIQLDDTAKEHPLIQNGRIDKVIHVHQQSRLWEFHLSPDELMPVMLYQTFMQQLLAFQQAIAQVSVQI
TTNTQTTFTTEQLTDYQWLALLNSQCNTPLVQKVLKTQTPQFEDRKIIILPVDNEAVIPYLKQQYLPITIEELYSYGFPPKPHI
EPKMDQQQAAAEVLKFFEEQKLEQAAAFQQAASLVKHEQMKEKQQQAPAFDGPRLGRNIPNDEPIIMPNGNILEEERRI
TIEGFIFDEKVRRLRSKRKILILKITDYTSSVVKFSNGEKDEQVFDATIQPSWIRVRGSGVQEDTFMRDLVMAQDLMEV
AHAPRKDYAPEGEKRVLEHMSNMSTMDATNKVGDVLAQAGKWKHKAIAITDHGGAQAFDAHAAGKAGVKILYGVANI
VDDGVPLIAYNEEHLTDATYVVDVETGLSAVYDTIIELAAVKMHKGNVIEFTFEQFIDPGHPLSQTTINLTGITDEMVR
GSKSEEEVLRMFKEFSEGTILVAHNASFDMGFLNTSYGKHGIPRAANFVIDTLELSRFLYPHFKEHLNLTLSKKGPNVLEQ
HHRAYDSESTGHLNWIPLKEAKENHDMHFHDDLNRHIGEGDSYKRRAPPHATILATTQAGLKNLFLKLISMSNVDFFRVP
RIPRSQSLKREGLLIGSACSNGEIPFAMMQKGVVEAKNRKAFYDIIEVMPKPVYAPLIEQELVKNEADLEBIIISNLVKIG
DELKGLVATGNVHYLNEEDAIYRKILVSGMGGANPLNRHSLPKVHFRTTDEMLTEFQFLGQDIKRIVENPNKQVADLCE
EVI PVKDDLYTPKIPGSEQBITDLSYNRARELYGDPLPEIIVEKRLKELNSINGNGFSVITYLISQKLVHKSNEGDYLVGSR
GSGSSSFVATMTGITVFNPLAPHYYCPECQYSEFYEDGSGYSGGDFMPEKACPKCGARLFKDGHDIPEFTFLGPHGDKVPDI
DLNFGSDYQAEAHNYTKVLFGEYVYRAGTIGTVADKTAAGFVKGYERDHNHLRGAEIDRLAKGSTGVKRTTGGHPGGII
VLPDYMVDVDTFTPIQYPADDQAEWKTTFDFHSIHONILKLDILGHDDPTVIRMLQDLSGIEPKTIPTDDPEVMRIEFGP
DVLGVDAEQIYKSTGTGLIPEFGTRFVRGMLBQTHPSTFAELLQISGLSHGTDVWLGNAEELIRRGAEANLAEVIGCRDDIM
VYLIHAGLDSGMAFKIMETVRKQGWNKIPDELRTTYLNAMKENNVDPWYIDSCSKIKYMFKAHAAAYVLMALRVAYFKVY
FPILYYCAFFSRAADDYDLVAMSQKRAVKARMKEITDKGMDASTKEKNLLTVLELCNEMLERGYKFGMIDLYKSDARNFV
IEGDTLIAFPRAVPSLGLNVAKAIVAAAREEQPFLSKEDLATRGKVSKTILBYMNENGVLKDLDPDENQLSLFDML

Seq ID 226

MERSNRNKKSKKPLILGVSALVLAAGGGYAYSQWQAKQELAEAKKTATTFNLVLSKQEFDKLPSVVQAEASLKKNGYD
TKSVVEKYQAIYSIGIQAEGVKASDVQVKKAKDNQYTFYKLSMSTPLGEMKDLQSYQSSIAKKGDTYQIAWKPSLIFPDMSG
NDKISIQVDNAKRGEIVDRNGSGLAINKVPFDEVGVVPGKLGSAEKTANIKAFSDKFGVSVDEINQKLSQGWVQADSFPVI
TVASEPVTELTGTAATKDTESRYYPFGEAAAQILGYTGTTAEDIEKNPELSSTGVIGKTGLERAFDKELRGQDGGSLVIL
DDKENVKKALQTEKKDGGQTIKLTIDSGVQQQAFATFDRPGSAVITDPQKGDLLATVSSPSYDPNKMANGISQKEYDAYN
NNKDLPTTARFATGYAPGSTFTKTTTGAIGLDAGTLKPDDELEINGLKWKQKDSWGGYFATRVKRAFPVNLRTALVNSDNY
FAQQTLRMGDKDFRAGLNKFIPEGEELDPLIAMPQAISNEDKFNSEILLADTGYGQQLLISPIQQATMYSVVFQNNGTLY
PKLVLDKETKKKDNVISAANAANTLATDLLGSVEDPFGYVNMYPNPNFLAAKTGTABIKDKQDTDGKENSFLTLDRSNK
FLTMIMVENSNGSATSATDISEPLIDYLEATIK

Seq ID 227

MKKVLMGVLSLGLLGAATGCTSDQEKAAKTKASSEKTEATSGASANGYTDPSSELKDSYDVVIVGSGGAGMTAALQAKEA
GMNPFVILEKMPVAGGNTIKSSSGMNASTKPFQKEKGIKDSNDKFFETLKGKGTNDQELLRYFVDESARAIWDLTGKIT
LSNLTITGGMSEKTRHPADGSAIGGYLDGLVRNVREEKIPLFVDADVTDLVEENGQIDGVKVKMKDDKEKTVKAKAVV
TTGGFGANEKLIQYKPELKNYVTTNQEETGTDGIQMIQKVGALVDMKEIQIHPTVQSDAFILGEAVRGEGAILASQK
ERFVNELDTRDKVSAANALPEKSAYLVFDQGVDRDRAKIDFYDQKGFVEKGETIBELAEKIGMPADTLKATIDTWNQDVN
AKDDQFGRTTGMEADLSTAPYYAIKIAPGIHHTMGGVKINTKTEVLREDGTPIKGLYAAGELTGGHGGQNRIGGNALADI
IYGRQAGTQSAEFASAQK

Seq ID 228

VIVRGGLDLAGTVIQKLWHVGFKILVLETECEPLAIRRTVSVCDAIFPQKEQVRVDLVAVRITSLKDCAKCWQKNLFPVVDQ
TASAIQQLKPLIVIDAILAKNLGTHRGMAPITIALGPGFSAPQDQDVVIVETMRGHRIGRLYFEGTALFNTGIPGEIGGKS
AERVVHAPASGQVTHLKNIGDLVLKGEALFLIDQVPVYSFLTGTLRGLISEKVTICYQLKCADVDPRPVKEVDCLTISDKA
RALGGAVLEAIFMIGRRKNVL

Seq ID 229

MYSIIVNGQSETCETNKKLMDFLREDLGLTGTDGNCQSGCAGTVLVNGKASKACLFLEKLAGKEVTTIEGLSQRQKDV
YAYAFKTAQVQCGYCIIPGMVISAOGLLNKPEPTKEDIQKAIKRGNICRGTGYVKIIEAIQLAAKMFCEBASIPEEHSNGK
LGEDFORVDAVEKTLGTGIYVDDIDIEGMLHASALRSAYPRAKVLISIDSTQALAHPCVAVFTAQDVFGNNKIGHLEBFISD
WDMIPGEITRYVGDVAVALVSKRKETLPEIKNLVEVDYBEMIPITSCEAALAEAPAIHEKGNILSHHILVRGNAAEVL
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QHHAALAAWLLKKPVKLLSREESLMVHPKRHGMEMDFTTGCDDEGNLTAMKAVIYADTGAYASLGGPVLQRACTHAAGPY
KYQTIDVEGFVAYTNNPPAGAFRPGVCQTAFAIESNLALLAEKVGLSPWEIRFKNVAVAGDTPNPGQLVS KNAALKREAL

AVKDVYEQAEVAGISSFTKNSGVGVGLPDTGRCIIISVEEGKIHVRTSAACIGQGMATVTTQIACETLNLPEMIVAEAPDT
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DKGKVAKFVAAYDMGQVVNPRAAEGQIEGGIANGMGYALTEKFALEGGYVKAQYATLGLVNAQTQVPIETILVHADNLHEG
LAYGIKGVGELATIPTAPALAGAYFALDQQLRPSLPLENTFYQKKR

Seq ID 230

MSDMPHPISEALLNWIFSEYQODGTIFGIRKPHYHADPTKTIISLFGEKMETPCGPAAGPHTQLAQNTILAAYLTSRPFVEVK
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DRYINEMQNAEGTPIWAEQAAAKKYLSTYFKVDDLYIEAISPKVCHSITLSTLHGCPSEIERIAAYLLSEKGLHSFIKC
NPTMLGYEYARQTMDELGFDDYMFDDHFKEDLQFEAEVPMQLRLQLLANSKNLSFGVKITNTFPVTIAANELPGDEMTMS
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LDKLAQVVEKAKTQARYQKSIKLPESKLRKTVPPLTDCYIAPCRSDGGCPINQDIPAYLRYVSEGNLYKALQVIVDKNPLP
FITGTICAHPCMTKTRQFYBESITHIREVKLEAAAHAYDELLTLEKPPKENAPKTAUVGGGPAGISAGYLLAREGMPVT
VFEKSETIGGVCSQIVPEFRISMESVQKDVQLAEFGMAEFTQGEAPSLAELKNQYTNVIYAIGAWKHGVLRLSEGRALN
SLEFLKANRENPTINPYBQIVVGGGNTAMDCAARAATLPGVKKVSVVYRRNKRNPADDEELTLALHEDGVDLFELLSPI
KHENQOLTCEKMLVGERDASGRRRPIGTGEMIDIPADTVIAAVGEKVDTEFYQALGIHTDNYGKVVSNQETLENTIPGVYV
IGDANLGPATTVKALADATKAANNICLVHNNHYEKDNLNSDVAFVRNKRGIILVTDMSCSQASRCLECSTICESCMDVCPN
RANIVVYVEGKQPIVHVRMCNEGCNCTFCPYASAPYKDKFTLFNSEADFYDSTNSGFFVQNPVDKICLLRLWGTVSTIQ
LTDQGLDVPEDLIALMVTMIREYAYCMA

Seq ID 231

MTKQTIKEQILHFMESQKKKSFSMBEIAQGLNLEKSSDFKILVQTTIAQMERESVSFNKKGKVLLEPMKDLLIEGTFRANER
GFGFVTIDPEEPDVIYPKEATNFAMDGDTVLIDVIOHADPPSDRGAEGKVKEIKERAVSQVVGGEFVAYSEBEAEMGLYGY
MIPKDKKLNQYTVTSIAPEGIKPVDGSIVLAEITYYPDQYPTSMKLANGLVKQVIGHKNDPGMDILSVVAHGIPTAFFPDEVLA
EADQVPEITIAESDLVGRRLDLRLQILVITIDGEDAKDLDDAVTVQKLANGNFFLGVHLADVSYYVTEGSQDMEAYERGTSVY
LTDVRVPMIPQRLSNGICSLNPHVPRLTMSCEMEITPEGEVLSHEIFQSVIQTTERMTYTAVNEILEEQPETLERYKELV
PMFKEMGELHHLBEMMRRAISFEDREAKVLVDENGHPKIDILLRTRGVGERLIESFMLAANETVARHYHDLKLPFIYRI
HEQPKKEKMQRTFFDFAAVLGLVKGTKENISPKDLQKVLQVENKPEEVVINTMLLRSMQAKYSEDNYGHYGLAAEYTH
FTSPIRRYPDILVHRLIRSYSQDQSEKNQEKWNEALPEIANHSSSMERRAVDAKREVDAMKKAEMVMDKVGETYDGISSV
TKFGIFVLENTIEGLIHVNMLKQDYFHFIEHNMALVGERTGMTLKGQKQVIRVEKADPETREVDLFELISAEVAPVEGP
KGRKKGKANSSTRSNNQRNKKDESFDGKKKKKKKGKGGKQPFYKAMKQKNNKKGKKK

Seq ID 232

MKNRYFILMILSFYFFAFGIETSAAEELRFSVETEIPENQIDKTKTYFDLMMKFDQEQILKVRAANSTDENLVIDVSVKSAT
TNSNGVIEYGESLTALDKSAPADLSEIIQLKDGGSVELPAKSEKAVELRVKMPKEFFSGQLAGGITFSEKVDFTKDKQKE
NTNGLAIKENRYAYTVAVLLRENETVVQPELSLEKVEPTQRNARSVISATLLNHEAAYLQSMKVNTANVNKKTNNVILEKEQ
EDMQMAPNSIFNFIPIYEBNEMEAGTYVLAMTVEGSGKKWQFTKEFTISKEAKTFNEKDVTVKKTESKLIYLLIGLLILL
LIICLFIIILRLKKQKNK

Seq ID 233

MSRKRKISLISLVIILVFTVGSAYFAVAGSYLKKTIDKGYVPIKNDYNEAQNKDSQSFLIMGLDNTIERKLGTTTRTDAMM
VITVNKTKKITYLSLPRDSFVQIDAKNYQGMQRIEAAITYDGTASVNTVEKLLNIPINHYVFNFLSFIKLIDAVGGID
VNVKQAFDQVTKDGGSIHFDAGKQHLDTKALSYAREKHSNDIMRGFRQOEIIQAVEDKLGSGQSIMKIMDIIDSNGN
IQTDVDSNELTHLVKEGLTWTNYDKQQLSPDWRTFSNEGRSMVELYPDSIENVRHQLRVSLNLEKPDERRDQDGYVFHTNGE
FLYQSDYTVQDRAAENEMTSINGNTYIGVPGNTQTGPLPSVKTENGFIK

Seq ID 234

MKKSQAVQLIQELSNANGVSGFETEVVRILQHATADFTIQRDLSIKNLYLEKKNLSEGFVVLFDHSDSEVGFMIQAIKEN
GLLRFPPLGGWVPNTISAQKVRIRNREGTYLPGVVTSRPPHMTPEERQRPPLTIADLTIDIGATSKEEVIETYKIDLGAPV
IPDVTCCEYNEQTDLFLGKAFDCRIGCACLVDMMEELKEETLPPKLAVTVAQEEVGERGALIAAKQVNPDLAIVFEGCPAD
DTAETPEMIIQSAMGKPMRLYFDVSMITNPEFQYALETAKIHKIPVQVSVRSGGGTNGMAITQVQAPTIVVGIFVRYAH
TPHCYVDFQDYQAAKELVLIQIKNLADAKIQALVQPLSKENK

Seq ID 235

MTTSQMIADFTTLAIQAGGWMELDRLYLQNRLLSMIGEQLGEVDIRPVATPAADLAEQLCQVASANQLVKTEQQKEQFMV
QLMDLLTPPPSVVNAFFAQHYAKEPQEATEYFYQLCQKNGTVIEQEEPVVFTSVYGDFLANKVHSEASKATLSAQSYPRCE
WCMATEGYQGSQFPATTNHRVIRNMNLDGESWGFSAFVQQAQYQQGVIAFEKHQSARSIKTFQQLLKIVFVPHYFAGID
ADFEQNEHVYQTLQFPPLAEASISEYVELANYPLINAGMVNVPVETFRLEGNASEVAQAANDIFBQWQMLKLPDEIQ
IVARRKELLYVMDLIFSRPQAKPSLTLAEVQGLTWNQKTALETVASAYQQLKEASAPAEETSEGKAAPLAMVAVPTH

Seq ID 236

MSILWINGILLVIVLAMVFNELYLKIMVKSAMLTBEEFKETMRKAQVIDVREKDTDFDAGHILGARSMPYSMLKTTIGS
LRKQDPVYLYDQKKALSIRAANLLRKNGYTDIYILKGGYDGTGKVKKRNS

Seq ID 237

VIFMNDPEPFIWKDLNAVDMGCIENELSEVLNPKRYETYYSIGRSGEFNETFNDYEPFDYIEIDVTIYPENLAACKRW
LTGKSKLITHNDEDKYLDICTMSKPTSPKNEGWVFTFNVBFRCPFRKRVNEQPKVIKTSIEITDHGDEIAPPYIEIN
SKGSDITLNGSNSLTILRTQSGIVTIDTEKGAIQEGNPLFTRGSIKTNPGQKLNISGNFIEAKFWNRSAYL

Seq ID 238

MSIHITFPDGA VPKFDSGITTFDVAKSISNSLAKKALAGKFNGLIDLD RPIVEDGSLEIVTPDHEDALGILRHSSAHLMA
NALRRLFPNIKFGVGP AIDSGFYDTONGESPVTAEDLPAIEAEMMKIVKENNP IVRKEISR AEALELFADDPYKVELITD
LPED E I I T V Y D Q D F V D L C R G V H V P S T G R I Q V F K L L S V A G A Y W R G N S D N H M M Q R I Y G T A F F D K K D L K E F I K M R E A K E R D H
R K L G K E L D L F M V S Q E V G S G L P F W L P K G A T I R R T I E R Y I V D K E I S L G Y Q H V Y T P I M A D V E L Y K T S G H W D H Y H E D M F P P M D M G
D G E M L V L R P M N C P H H M V Y K N D I H S Y R E L P I R I A E L C M M H R Y E K S G A L S G L Q R V R E M T L N D G H T F V R P D Q I K D E F K R T L E L
M V A V Y A D F N I T D Y R F R L S Y R D P N N T D K Y F D D A M W E K A Q A M L K A A M D E L D Y F E A E G E A A F Y G P K L D V Q V K T A L G M E E T L
S T I Q L D F L L P E R F D L T Y V G E D G E N T H R P V V I H R G I V S T M E R F V A Y L T E V Y K G A P T W L A P I Q A T I I P V S V E A H S E Y A Y E I K
E R L Q A Q G L R V E V D D R N E K M G Y K I R A S Q T Q K V P Y Q L V V G D K E M E D A T V N V R R Y G S K E T S V E D L S I F I D S M A A E V E N Y S R

Seq ID 239

V S N K K V A G T I I L N L N D G S K K F L M H P I G E A I E F A M A K V S D E M T G L A S M L Q W P K E E V Q L D V T S I S L V E L T N A H I N K E N V P L F V
F E M D E Q A L K N E M D K D Y T W V A P A E L K T I W S K Y H I E G V M F

Seq ID 240

M K K F D G V I F D M D G L L F D T E L I Y T S T Q K V A D A M G L P Y S K E V Y L D Y V G I S D E E V Q E N Y R R I Y A S Y G H D T V E E F I R R S Y D D T L
Q E F R S G N V E L K P G V V F L D F L D D Q K I P R L V A S S N V R P A I E M L L S H A G I Q D R F V G I V S A E D V K R A K P D P B I F Q K A R Q L L G T E
A P K T L I F E D S F H G V S A A H S A G I P V I M V P D L L Q P T E V I Q E K T L H V L E S L H Q A P H Y L K

Seq ID 241

M N E L V F L H S Q Y I E E B P Y T D E V I A K Y S Q I K R E S V S K L I K Y Q K D L E E F G K V G F E I R A M E S G Q K A K F Y Q L N E E Q A T L L I T Y L
D N T E P V R R F K K A L V R Q F Y D M K N G L Y A R M E R Q K E K S V R K S M T D V I K E L G L S P H Y Y K H Y T D L V Y K T A L G F N A K Q L R E A R E V S
K K S T I L D F L T S B E I E A V N K R E Q Q V A T L L T L K M D Y D T I K S I L N G Q G V L Y Q T T L K M P V A T N

Seq ID 242

M P K P G E T I H A I E H F T A G G G K G A N Q A V A A K R S G A E T Y F I G A V G N D G A G A M M T D L M S Q D E I N L T G V T T L E K T A T G Q A F I M V D N
A G E N S I M I Y A G A N N A F T P K Q V Q E H Q E I I E K S D F V I A Q F E S A I D S T I A A F K I A K K A G V K T I L N P A P A L E Q V P E L L N V T D M I
V P N E T Y D I L T G I K I T D E A S M R K A A E A L H Q L G I E A V I I T V G S K G A F Y D V N G R S G I V P A F K V A V D T T A A G D T F T G A L S S I L
E K D F S N L E A I R Y G N K A S S L T V Q R F G A Q P S I P Y Q H E L A D K

Seq ID 243

M I K L A H I H K Y Y Y S E E T L H V L D D I N L Q V D A G E F L A I M G P S G S G K S T I N L L G F I D E K K F E G T Y L F E D R E I G D F S D K E L S R I R
N E A V G F V F Q N F S L I E T L T V E E N I E L P L L Y S G L T P K E A K R D R V E V L T K V G L P D K G K K H P K Q L S G G Q Q R V A I A R A I V N R P S F
I I A D E P T G A L D S K T S E E I L T L F Q Q L N N E G V T I I L V T H D E E T I E Y C N R L I K V R D G K I L E E V L T

Seq ID 244

M K K S G Q K K R N K V W F G I G A A V V V V G F I G A K T V F S S K E V E P Y T T Y T T I T E M A S L K L D G Q V S F L D T R D I F F D P S L G K I A B I N V
E N G K E V K D S P L L T Y N N S D I Q A T E T E Q A N A V N R N L Q V Q A Q E N V N L A T Q K Y N E A L N K V A A A K Q K L N T A K A E E K E T L N A E
I Q Q L N E A V S A A N S E V A Q A N Q A L Q L A N S D A V G A A N T L E Q T R G K V N T V V T A P I D G Q V T D A S A M S S T D K P V I K I A T Q K K N I Q G
K V T E Y D Y D K L Q T G E E V T T V T V G S G K S A P G K I V S I A Q T P I A K N E G N P V V S Y Q F T V E G D F P W A E G L S T S I A V P Q K M I I P T A A
I Q K E G Q K E F V Y V Y K A G K A K T P I E T E T N L G R K V V K S G L N W K D Q V I A N P N K E L K D N Q D V Q V A N D

Seq ID 245

M I K I I V V V A F M L T G F S L T A M S A S A E E I T D L F L Q K E V T Y S G V E G G K I G E N W K Y P Q F V G E K A V D G D E T T R W S A D K Q D E Q W L I
V D L G E V K N I G R L V L Q L H A E S P V Y E I L V S T D G E S Y Q S I F K E E N G K G G Q P T K K Y I D G N N V Q A R F V K Y Q M K M W Q H T N K Q F Y S S
S I I S F E A Y E K R L P R A I K L L T E N L T I S E K R K Q Q L A F E V S P A G V D I T E D Q I E W S S S D P T I V T V D Q T G N L T A V K S G E A K V T V K
I K G T E I S D T I P V T V A E N K Q Y A E M R A K W K M R L L G T T Q V D N D A D V Q Y R A Q I A T E S L A L W Q T L N Q A A D R E Y L W E R K P S D T V S
A D Y T T Q F T N I K K L A L G Y Y E P S S E L P E K P E V Y D A I V K G I E F M I D T K K Y N G T Y T G N W N D W Q I G S A Q P L T D T L I L L H D D L L N T
D A E K L N K F T A P L M L Y A K D P N I Q W P I Y R A T G A N L T D I S I T V L G T G L L L E D N Q R L V Q V Q E A V P S V L K S V S S G D G L Y P D G S L I Q
H G Y F P Y N G S Y G N E L L K G F G R I Q T I L Q G S D W E M N D P N I S N L F N V V D K G Y L Q L M V N G K M P S M V S G R S I S R A P E T N P F T E F E S
G K E T I A N L T L I A K F A P E N L R N D I Y T S I Q T W L Q Q S G S Y H F F K K P R D F E A L I D L K N V V N S A S P A Q A T P M Q S L N V Y G S M D R V L
Q K N N E Y A V G I S M Y S Q R V G N Y E F G N T E N K K G W H T A D G M L Y L N Q D F A Q F D E G Y W A T I D P Y R L P G T T V D T R L A N G A Y T G K R S
P Q S W V G G S N N G Q V A S I G M F L D K S N E G M N L V A K K S W F L L D G Q I I N L G S G I T G T D A S I E T I L D N R M I H P Q F V K L N Q S D K D N
S W I S L S A A N P L N N I G Y V F P N S M N T L D V Q I E E R S G R Y G D I N E Y F V N D K T Y T N T F A K I S K N Y G K T V E N G T Y E Y L T V V G K T N E E
I A A L S K N K G Y T V L E N T A N L Q A I E A G N Y V M N T W N D Q E I A G L Y A Y D P M S V I S E K I D N G V Y R L T L A N P L Q N N A S V S I E F D K G
I L E V V A A D P E I S V D Q N I I T L N S A G L N G S S R S I I V K T T P E V T K E A L E K L I Q E Q K E H Q E K D Y T A S S W K V Y S E A L K Q A Q T V A D Q
T T A T Q A E V D Q A E T E L R S A V E Q L V K V P T K E V D K T N L L K I I K E N E K H Q E K D Y T A S S W K V Y S E A L K Q A Q T V A D Q T T A T Q A E V D Q
A E A K L R S A V K Q L V K V P T K E V D K T N L L K I I K E N E K H Q E K D Y T A S S W K V Y S E A L K Q A Q T V A D Q T T A T Q A E V D Q A E A K L R S A V K L T L K N S G E N
K K E Q K N G N N G H L N T S T G V D Q T G T K Q V K P S S Q G G F R K A S Q F L P S T G E K K S I A L V I I G L L V I A S G C L L V F R K S K S K K

Seq ID 246

M K K S V L F T S L I V L S S L A L A A C G G G S D D K G A S N G G S D N Q V Y T M V E S Q E M P S A D P S L A T D E V S F T L N N V Y E G I Y R L D K D N K P
A P A G A E K A T V S E D G L V Y K V L R E R S K W S D G K P V T A A D Y V Y G W Q R T V D P A T A S E Y A Y M F E P V K N A E K I S K G E L P K E E L G I K
A I N D H E L E I T L E T A T P Y F D D L L A F P S F L P Q R Q D I V E R F G K D Y T K S S D K A V Y N G P F T L T E F D G P G T D T K W S L T K N E Y W D K E
T V K L D K V A I N V V K R A P T A L N L Y E T G E V D D T Y L S G E L A Q Q M Q N S P D L V Q L K A A S F Y L E M N Q A D E K S P L T N A M L R R A M S Y A I
D R D S L A K N I L A N G S L P S Q G F V P V D V A K S P K T G E D F V K E A G S D K L V K Y D K K K A V E Y W N K A K Q E L G V S N L T V D L M V D D S E G A K
K M G E Y L Q G S L S D T L E G L K V T V T P V P M A V R L D R T L E G D F Q I A V R G W S A D Y S D P I N F L D L L E S S T S N N R G R Y S N P E Y D K F I A A
S K T T D V N D P E K R W E D L I N A E K T V I A D M G V V P I Y Q A E S H R A P N V K E I I Y H P T G A K Y D F K W A Y K E

Seq ID 247
MSKKPSGYQKKVLTVLLVSFGFGLLAQFSPMKSGGFSNFDSDYVKASABSSKTKBPAPVKIEKKVKPLSYGQQVNVQRIE
KKQYDGHLDLPLELQTDAKWKDTAYGFGNVDPNTIEINGCAIVSLAMVGSYMDHQEVFTPLDVLAWAKNDFMBSGGTAW
IFSAAYAEKMGYNQCEIGDIETVAFLKEGHPVVISVKPGYFTTTGHIMVMSGVDEKGFWDNDPNSKEKGHSKRTFTABE
VMNEALNFWAFY

[illegible]

seq ID 249
 MSSLLKRLVQLVLLVVAVLLIRHVVFSPAAVNGSSMEPTLHNDRLWVTSIKKQRFDIAPFSPRNGQVAKRLIGLPGE
 TVEYRDDTLTYINGVSLSEDYLA SAKRNVSKENYNTQDFTLETLEATQSLTVPEGMFVVLGDNRP RSDDSRYFGFVKQASVE
 GVLTFRYYP LDKIGFP

Seq ID 250
 MANDDLQQRLDKMGYGTPLVNPPEEHQKYGTFRRERCRLSMTVAEMKDAQNQKHLLEELAKHPFVTVLLNGEISSDLQSTYI
 KLLNKGANFKVNNFVENNPDSLGLLLAEKHAVDEPVIDVTEKYPQATETFPKEEPTAKKSFQKQLFHS

Seq ID 251
VYLKRIETIGFKFSADKTIIEFEDDVTAIVVPGNGSGKSNITRAVRVWLGEQSAKNLRGGKMNDIIIFAGSEGRKPLNIAEVT
VTLDNSDEHYLALDYSEISVTRRLKRTGESDFFINKQACRLKDIQLDFMDSGLGKESFSIISQKGVEAIFNSKPEDRRGIFE
EAAGVLKYQKQRKKABQKLFETEDNLSRVQDIIYELEDQLVPLAAQDAKKYLALKEELTEIDVNLVTVEITQEAKAIEWT
KTQELTAIEBKLAGASQVHDEGLEGLNLRSKRNLDEQIETEQOQDLQVTEALQKAEGQKNVILRSKHTSQTASEYBET
LBETASKIVRYRBEELQTLETAIAEKTAQRQTLKEALALATKDVEKYSKSSKELMEELRSQYVVMQEQOANTANDLKYLERQ
YQOETAKNQQSIAKHAEBEQMVSAALAMKETLEKQKVAKQGLQEYLEYTALKATLEAKRRIHQARQNDMYQAMNQVQQA
KARQSKLOEIENYAGFYGVKAVLRHKNQLTGIVGAAELIEVPKEEYLAITALGGAQHIVVENEKDGKAGITFLKQ
HSGRATFLPLTTIKPRSVSAMNRLAGAPGVGASLVRYPEQVQVTIIONLGGVTIIAADLTSANQLAKLVNYQYRVVS
LEGDVMMNPGSGMTGGANKRNGOGSFLSQAQELQITTEQMTQELQRSVQEQVQALSQEVKTTATERAEMLRSAGEONRLKQ
QEIDNKLVAQNTBETITRLTKKKRLFEYESRESLHQFLTETQTKATLEQQANLTATERLDAEMKQVEQEASMTFFKAQAQ
ERLTTVQAEQVAAEQCAHFARQKQDQEQDLDELLIRETAIRQQLQGLSSSHSDHQLTEGGLAAQVQALAEKQTALQTSLO
TARSQRQALQBEVDDELDTKLAENKRRQQVYLADKTQIEVLKNRAEMQLDSSLSYQLQEYSITFEAAEYAYFPIDDLAQAAQ
TVKRLKQBIERLGPVNLSAIEBQEQVDERHQFLVSQRDILLNAKEQLFETMDMEDQEVKREKVFVEAIRGQFKVVPFNM
GGGRAELVTNPDELNTGIBIEAQPPGKQLHLSSLSSGERALATAALLSIIRVVPVFCILDEEVALDEANVARFGH
YLSEFEDQTFIVTVTHRGKTEAADVLGYVTMQESGVSKIVSVRLIEVKEGGAIEKSN

seq ID 252

MQKVAKVIVDVPTMQTDQPFYTLVLPENLNEQLAVGMREVVPFGNGNRHVQGFVLAIEFVAATVLDETNVQLKELVAVILDLK
PVLNTEMLALADYMKETFAFKITCLQTMPLPSVMRADYQKYIYLTDELSEELQDQLFFYGLEEISWDQAQERGLLPQLMALR
KQKQVDIRXEVTTRNKVKMVRPIQAAKEFEQLBEETRLGLRGKAAKQQLLYYLQRLGETKVTAVKMEKELGFSSTALINEAA
KNGWLTFIEKEAYRDPFANQTFEKTHTALSNAEQVAVETILQSVOEQSQSYLTLEGITGSGKTEVYLQVIAEVLNQGKTA
IMLVPFISLTPQMVRQKFRSPKRGVAVHMSGLSQGEKQDEWRKIERGEAEVYVVGARSALFAPINIGVITVDESEHSAKYQ
EETPRYHARDLAIWRSSEYHCPVVLGSATP\$LESRARAKNVYQRLRLTQRANQAATLPTIDVDMRQEVENGNVSSFMS
LQEKLERLEKNEQSVLLNRRGYSSFVMCRCDGXYVLPFCNCDISLTLHMSDKTMCHYCGHEERIPIYRCPNCQCKIRIYF
GTGTQKVEELQTLTLPDSRIILMRQDVITRRKGAEKILARTYBEGQADILLGTQMIARKYGFNVNVLGVNLNATLNLDPY
RSSERTFQALLTVQSGRAGRAEKPGEVITISFNPEHYAIQLAKAQDYEDFTYKEMIRLHRGDYPPYFTYQITASHPENEA
AKQMFQIQLTKQGLSPQAILGPTPNAIMRVNNRYFYQVITIKYQBPMLQPLLKEILTDTQRATARGLKLSIDAEPNFI

Seq ID 253
MDLHLTVDLKKTTLIDLADQAHLLIEQTTLKPKKEAAILFLLLDNLQVALGLAKEKATYKEVSFDHSLKKPTWDTLAY
LAQAFLLDDAKESGLTLBATPTTVKIPKSAVATVTNYKETVTFILERFGYSLFKKPAPKARPAPKARHKKTKEVSQIPFYID
TRQSKATVFWQKRNEMLLKAGATMMPEAPLNKDGSGVGFSAARFGEKLRDERKGQFKDFVTTTIDVLKSVNEVGLFLYFAGTN
SWLELVDENGKTLNEWTVVE

Seq ID 254
MNRWKVYATVLAACMLFGWIGVEAHASEFNFVAVTPTIPENQVDSKTYFDLKMAPGAKQTVEIQLRNDTDEDITIENTVNSA
TTNLNGVVVEYGQNGIKPKDKTLRFNLKDYVEAPKEIILPKHSQKTLPLTTIMPKDSFDGVMAGGITLKEKKKETTTSADQSK
GLAINNEYSVVVAIILQONNETKVPDLKLLGVKPGQGVNARNVINVSLQNPQAAYLNLQLHLINTVSKGGFTLYQSDTBDMQV
APNSNFSYPISLKGERLTPGKYVLKSTAYGVKDEKGTQYQVKGANGEBRYLYLWELTKFTISLQDVAKELNEKDVTIKGTNW
VLYLLIALIILALLIIFLYLRKKKKEEQSEQ

Seq ID 255
MSKNFWATLPKPFVFLAPMEDVIDVFRHVVKAGAPDVFFTEFTNSDSYCHPEGKDSVRGRLVFTFEDEQPMVAHIWGDKP
EFFREMSIGVAEMGPGQLDINMGCPVNPVAERKSGSGLILRPEVAAILDAARAGOLPVSVKTRIGFTMAEMEAWITHLL
EQDIANLSIHLTRKEMSEKVDAAHWEVIPQIMAIRDRVAPQTITINGDIPDRQKGLSLAEQYGVGDGIMIGRGIFKNPYAFE

KBPKTHTPQELLGLLRLQLDLQDKYAEVPRISIVGLHRFFKIYVKGFFGASDLRAQLMNTKSTDEVRQLLATFETEHGVLD

Seq ID 256

MRYLDARAIENIATGAFLGTGGGGDPYIGKMMALSAIBENGPFVLVSPEBIAAEDFFLPAAMMGAPSVAIKFPKGFDEFV
RVFEKLGKYLDOETIAGTFPMEAGCVNSMIPIVVAAKLGIPLVDCDGMGRAFPPELPMVTFHNLGMSATFMAITDEKGNIGI
METIDNTWTERLARVQTVEMGASALVSIYPATGKQLQDYGHNIVTLSEBICKVIRGTIADQEKQALVSEVTDGFELFQGG
KILDVEREVKGGFNLGRVKLSGLNSDAGSEAVVHFQENENLIARKDQVLIAMTDLICMVDLETLPVTTESLKYGKRVQVM
GLKANAARWTKKGIETVGPFRYPGYEMDYQPLENLVAKEDK

Seq ID 257

VTIVLLILLINVSIMFLLGQLLSSENSPLAGVIWATKDAVAKELNTDLFWSWNKFVLPFIFFLVDAAVLYWRLIRRYHOMQLRH
IISELHYIADGNYNHRIPFELSGDLAKVVTSLNGLVDSTVAIAEDERRRIEKSDELITNVSHDIRTPLTSIIIGYLGLEHDR
QPHSQEDLLKYTHATYVKAQMKLLVDLFEYTKVRQSPVPIHTTTFDMAQLIEQLAADFELEAKKINMQIQVKANPASIM
MEGDTKLVRVFNLLSNALKYGGGHHIVMEVDKVGTEAIAVRNDGPAIPKHSLDQLFDRFYRVESSRSQFTGGTGLGL
ALAQSVIALHGGYIYAKSDQKWTSTFIHLPLQRTNKKSES

Seq ID 258

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Seq ID 259

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MACRMSLVIAPLVRGRIPTIVEQVNTVVTPTGTSVDVVVTEVGIAINPKRDTLIDCFKTLDPVQFTLEELKDKAYNIVGTPE
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Seq ID 260

MTMNPFGKQFQDQDVIIIVAGYILRYNLSYREVQEIYDRGINVSHIT
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Seq ID 261

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Seq ID 262

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Seq ID 263

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Seq ID 264

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Seq ID 265

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Seq ID 266

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Seq ID 267

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Seq ID 302

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Seq ID 320

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Seq ID 321

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Seq ID 322

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Seq ID 333
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Seq ID 341

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Seq ID 342

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Seq ID 343

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Seq ID 355

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cataaacagt	ttggtgagcc	gaaagcaatt	gtgacggata	aagcaccttc	tcttggtctc	420
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aaaaagaacc	gaagaaatgg	aacgctcttc	ggcttttcgg	tgtctactga	aatcaaggta	660
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Seq ID 357

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ELAKA

Seq ID 358

MIKKEIIFVPKRLVKKEIDLLVENLTTIKDDNGEFLDDFDGLKVDDKSWTVWNWPQGVGLYGIYKNYQMTFNPRAYQVVNE
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RKLLIETLEAQVRALKTYQSESGLWHTLLDDPSSYVESSATAGFAYGILKAVHKRYLPQEYKEVAYRAIQGLLEQIDEKGE
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Seq ID 359

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LADKLSSEGLNAHPSDNTHYSIYRKACVNGTMMGLCTILDVNMALGKGTSTAHKMVATIVNEFAKVAEVEKIELDVPEVIA
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Seq ID 360

MTFFPYSEGNKRYHSWNYALRNEFGGKIFKVPIDGGFDCPNRDGTVAHGGCTFCSSVSGSGDMIVAPEDPLPIQFQKEIDMMH
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SKLINRAHDYQTYLDGVAKLKRNIRVCTHLINGLPGESLEMMKENVRRTIILSDIQIGIKLHMLMRKTRMLRDYHEGRIL
QLMSRPDYVNVICDQLEMI PKEI I I HRLTGDAPWDSLIGPMWSLKKWEVLNAIDEELLRRDSFQGYDVRRKVS

Seq ID 361

MAKTGMYVGLDIGTTSVKVVVAEYIDSQMNIIIGVNAKSEGINRGIIVDIDKTQVQAIQRAVRQAEKAGIQIKGVSVGLPA
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VLNTSFNABALKINYGDAPERTSPDEEFFVDVIGQSEPVKVDERYLSEIISARMEQIFNKAKDALDQIEALELPGGVVL
TGGAASLPGVVDLAQEIPIGVNVKLYVPNHMGLRNPVFTNVISIVDYSANLSEVYQLAKIAVGETSAAARQMVEQETTNTY
ESYEAPETTYDEPEPKESGENVNKIKGFFTNIFD

Seq ID 362

MAKKTIMLVCSAGMSTSLIVTKMQKAAEEKGMEADIFAVSASDADNNLESKNVDVLLGPPQVRFMKAQFEQKLAPKGIPLD
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Seq ID 363

MKNHCKINVMGLVFLILPLLTNSFGAKKVFAEETAAQVILHCKMTDLPDPLIONSCKEMSEFDQYQGLADISFSVYNVT
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QTDGPTVSDPNYIPSLTPGGTLKFVYVYHMLNEKADPTKGFSNQANVDNGHTNDQTPPSVDVVTGGKRFVKVDGDTSDQT
LAGAEFVVRDQSDSTAKYLSIDPSTKAVSWVSAKESATVFTTSSNGLIDVTGLKYGTYYLEETKAPEKYVPLTNRVAFTID
EQSYVTAGQLISPEKIPNKHKGTLPTSTGGKGIYVYIGAGVVLILLIAGLYFARRKHSQI

Seq ID 364

MNNRIETLEFEKVKQMVRFVVTAAQGKEELAEVFPVSEKQTTITNLQETEDGLKVQRLRGGIPIPKLENIRPHMKRIEIG
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NEKSLVLFDELGAGTDPQEGAALAIAILDDLGKKSAYVMATTHYPELVYGYNRANTINASMEFDVDTLSPYRLLIGVPG
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VTYTGQRGTLRLKNGNQWQVEIGILKMNVSDELTPVAPQKEPTQRVIHAVRSESSSEVPNQDLRLGRKRYEALSBDQYL
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Seq ID 365

MFDGYCSRVVNGNPNVKPATRKVLEVIDRLDYRPNVARGLASKKTTTVGVIIIPDVSNMFFASLARGIDDVATMYKYNII
LANSQNDQKEVNVNLLAKQVDGVI FMGHHITDEIRGEFSRSKTPVVLGASIDPDEQVGSVNIDYVSATKDAVNKLAKS
GNKKIAPVSGALIDPINGQNRKGYKEALSENMLPYSEGLIFEAQYNFKDGLSLADRIHNSGATAVYVSDDLAIGILDGL
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Seq ID 366

MKKIDILSRKKNAGVIAVVRGKSKKEALNACHAI I KGLTGIELTFTVPOADQVIKELLSFYKDQPEIVIGAGTVLDAVTA
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GVSLDNMKEWFDAGVVTVGVGNNLLAPATGDFDKVTEVAQQYAAKMKEIKR

Seq ID 367

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ELRETYLSAMKENNVDPWYIDS CSKIKYMPKAHAAAYVLMALRVAYFKVYFPLIYCAYSVRADDFDLVSMCKGKDAVK
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Seq ID 368

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Seq ID 369

MIKITFPDGAVKFESGTTTLAIAESISKSLAKKALAGKVNGLVDLTRPIEEDASIEIITPDHEDALGLVRHSAHLMQA
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KLGKELDLFMVSPHVGSGLFWFLPKGATIRRTIERYIVDKEVSLGYQHVTYTPIMGDELVELYKTSGHWDHYQEDMFPPMDMGD
GEMLVLRPMNCPEHMMVYKNTIHSYRLEPIRIAEHLGMMHRYEKSGLSGLQVRREMTLNDGHTFVRPDQIKDEFKRTLELM
VAVYADFNITDYRFLSYRDPNNTDKYFDDAMWEKAQAMLKAAMDELELDYFEABGRAAFYGPCLDVQVKTLALGTEETLS
TIQLDPLLPERFDLTYVGEDGENTHRFPVITHRGIVSTMERFVAYLTVYKGAFTWLAPIQATTIPVSDAHGDAYEIKE
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Seq ID 370

MTTNFWKELPKPFFVLAPMBDVTDVVFRHVVKHAAAPDVFFTEFTNSDSFCHPDGKDSVRGRLTTFTEDEQPMVAHIWGDXP
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HQDIANLSVHLRTREKMSKVDADHWEVI PKI IALRDQIAPQTLITINGDIPDRQTGEELAEKYGVGDGIMIGRGIFKNPYAFE
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SEQ

Seq ID 371

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DSTVAAIEDERKIEKSDBLITNVSHDIRTPLTSIIIGVLIEDGQYHSEEDLLKYTHTAYIKAKQMSLVDDLFYTTKVR
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Seq ID 372

MNHFKGQFKKDVIVAVGYLRYNLSYREVQELLYDRGINVCHTTIYRWVQYKSVLYYLWKKKNRQSFYSWKMDITYIK
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EF0008

Seq ID 373

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EF0028

Seq ID 374

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EF0146

Seq ID 375

ATGAAAAGATCGCAAGTGCAGGGTTAAGTATTTTAGTCGCAACGGGGTAGCAGGTATTGGGGGAAATGAAGTACAGGCA
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AAAGAAAAGACACCAATTGATCAATCTGTTAAAGACCAACCAAGCAGTGGTTCGATCAAAAACCAAGGTGCATTGGATCAAAGT
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EF0153

Seq ID 376

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EF0394

Seq ID 377

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EF0443

Seq ID 378

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EF0568

Seq ID 379

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TTA

EF0591

Seq ID 380

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GTGATGGCT

EF0592

Seq ID 381

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EF0658

Seq ID 382

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EF0727

Seq ID 383

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EF0775

Seq ID 384

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EF0779

Seq ID 385

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CAA

EF1091

Seq ID 386

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EF1752

Seq ID 391

ATGAAAAGAAAACTAA CAAAATCTCCGAATAACGTGGTTTTTAA CAGGAAGCTTAGCTGGGATTGCTGATTGGCTAGGAATT
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EF1753

Seq ID 392

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EF1791

Seq ID 393

ATGAAAAAGTATTTAAAAATCACAATGGTTTGTATTTTATTGGTAGGATTTTAGCTGGGTGTACCAATAAAAAATGAAAAAT
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EF1800

Seq ID 394

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EF1818

Seq ID 395

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EF1850

Seq ID 396

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EF1877

Seq ID 397

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EF2174

Seq ID 398

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Seq ID 399

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Seq ID 400

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BF2704

Seq ID 401

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BF2713

Seq ID 402

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BF2802

Seq ID 403

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TAT

EF2813

Seq ID 404

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EF2820

Seq ID 405

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EF3082

Seq ID 406

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EF3256

Seq ID 407

ATGAAGTGAATAAATTTGTTAAAGGTTTTCGGCAATTGCATTATCAAGTTTAGTATTAGCAGCATGTGGTGCAGACAAG
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EFA0021

Seq ID 408

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EFA0044

Seq ID 409

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EFA0052

Seq ID 410

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EFC0004

Seq ID 411

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EFC0012

Seq ID 412

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EFC0021

Seq ID 413

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BFC0053

Seq ID 414

ATGACCCCAAGTACATTTTACACTGAAAAGCGAAGAGATTCAAAGCATTATTGAATATTCTGTAAAGGATGACGTTTCTAAA
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GAAAACCGACAAAGTCAACGAATGGCTATTATGAGCGCAGCTTTACGACACGTGTAGGCACGCTAGAAATTAAGTACCC
AGAACACGTGATGGCCATTTTACCCACAGTGTGTAACGTTATCAACGAACGAAAAAGCCCTCATGGCTTCAATGTTG
GAAATGTAAGTATCAGGCGTTTCAACTCGTAAAGTATCAAAATTTGTGGAAGAACTTTGTGGTAAATCCGTCTCTAAGTCC
TTOGTTTCTAGCTTAACAGAACAGCTAGAACCTATGGTTAACGAGTGGCAGAATCGTTTATTATCAGAAAAAATTTATCCT
TACTTAATGACCGATGTACTCTATATAAAAGTACGAGAAGAAATCGAGTACTCTCAAAAAGCTGTCTATAGCGATTGGA
ATAACCAAGATGGCGACCGTGAATTTATCGGCTTCATGATTCAAAGTGGCGAAAAGCGAAGACAGCTGGACAACATTTT
GAATACCTAAAAGAACCGCGTTTCAAGGTACGGAACCTCGTTATTTCTGATGCGCACAAAGGATTAGTCTCTGCCATTAGA
AAATCCTTACCAACGTAAGTTGGCAAGATGCCAAGTTCATTTCTTAAGAAATATCTTTACCACTTCTTAAAAAAT
TCAAATCTTTAGAGAAGCTGTAAAGGAATTTTAAAGTTCAAGATATTAAGTTAGCGCGTGAGGCTAAAAATCGATG
ATTCTGATTATATCGATCAACCAAAATATTCAAAGCTTGGCATCATTTGGATGATGGATTGGAAGACGCTTTCAATAT
ACGTACAGGAATTTCCCAATCGACTAAAGAGTACCAATCTAATTGAACGACTGAATCAAGAAGTACGAGAAAGAGAA
AAGATTATTCGCATCTTCCCAATCAACATCAGCCAATCGCTTAATTGGAGCCGTTCTTATGGACCTACATGATGAATGG
ATTTATCTTCAAGAAAAATACATCAATTTTGATAAG

ARFC0021.1

Seq ID 415

ccaagtgaaggggaaaccaaggaagggttgaagagaagcgagcgaaggaagtaacgaaccacgttaacgcaggaagatac
actgaa

ARFC0021.2

Seq ID 416

gagaacgaattcaagctgcacaaattgggtttacattcttttctcgatcgggtacaccaatcggtgggtctgggagtgagg
tctttgagcagtagataaagaaaaatgcaaccattaccacagacagagaacaaagcaggacaaggctggccaggcaatg
cgtatgcgcagggaattgtacttgggtatgtgttaatcggtttgacacgt

ARF0324

Seq ID 417

ctcaataaagggttgcgaaagtagaagctgtttacgatcaagaaatccaagaggtgaagagaacgtacaatgcgattat
tgcaaatgtagaaagagtgctgc

ARF1627

Seq ID 418

gtgctacattgcttacaaccaaactgtcggtatgccagaagcagcgaggacagttatttcaaatattcacaagggtggga
caccggtggtgaagcaggggctacattgcccgaattattaaaaaatgt

ARF1650

Seq ID 419

aagcggctgcaaaaaagctgaaaaagctctatctcgagccgatccggaccgagaaggtgaagcaattgcttgccatt

CRF0097

Seq ID 420

atggcattgttacctaattgtcacaagtaaacagccacgggtgttgccatttctgtcacaggtacttttaagccaaagaag
gtcactacaatgccactaacatttctgaaaaataattcggtgcg

CRF0257

Seq ID 421

agttgcgcatthtggcagtttctttcggtggcaaatattttcgtagtccagctactggtacgaccacgattcctgcattc
gctgtcatcaggaatttaacgcactcactcgatctgcgcgcgttctggtgaagaaaaggccatttcggcggaataacact
tcatctactggaaataga

CRF0635

Seq ID 422

ggaagcatcgtagataattcaccagatatcaatgcgattggaccagcaagaataagaccgcgaaaatttgt

CRF1152

Seq ID 423

agccaggggcaatgttatggccaattaatgctgcttcaatacaaatgttccagaaccacaaactggatcataaaaggac
gatctttgcgccaattgggtcaacatcactaaagcagctgccatgttttct

CRF1720

Seq ID 424

Tcatttcgtccagtcctcctactgttcttcgtacgtgcgttttccggtataaattcacaacattcctaagacagtt
gctaattgccattgaagacaattcacaagttcctactttgaagactaagcccaatgccgatgactaaaattactgaagca
atcaataaattctttttcttatcaaaattaatcttattatcgattaaagattttcacaaccacttgcggcaatcacaccgaac
aagacgaactaattcctgaaataactggtcctgggatacttagaatcaaggcacttaattttccaacaagcctaagct
acagcgaacaccgcggctccaccaatcacgaagacactgtgtactcttgtaattgctagcacgccgatattttcacca

EF0008

Seq ID 425

MINNVVLVGRLLTDPDLRYTASGSAVATFTLAVNRNFTNQNQDREADFINCVIWRKPAETMANYARKGTLGVVGRIQTRN
YENQGGQRYVYVTEVVCENFQLLESRSASEQRGTGGGSFNNNENGYQSONRSFGNNMSSGFNNNNNSFNPFSSSQSNNNGM
PDFDKSDPFPGSGSSIDISDDLPF

EF0028

Seq ID 426

MAVKKKKATFWBFFOGLKTFMLPVALLAFMGILLGLSSFSSESMIETIPFLGKPAVKIIFQFMSTIGGFAPAYLPVMPFA
MAIPLGLVRKEKGIAAFSGFVGYTVMNLAINFYLVQTNRLVDPEQLRREAGQGMVFGIQTIEMGVLGGIAGLIVYKLNHRF
YTVQLPDSFAFFSGARFVPIITSLVMAFVGLVPLVWPLPALMIMAIGQLIQRSGIFGPFPLFGSGERILLPFLHHILVSM
IRPTEAGGSAAVAGKEVFGALNIFYAELQNNLPISPSATAFLSQKMPFTIFGLPAASLAMYHTAAPANRHKIKGLLLSGV
IATAITGITPIBFLFLFISPLLWLFHVIMTGLGFMMVALLGVVIGNTDGGLLDFVIFGLLQGTYYKWWVLIVGAIWVWV
YYVFFKTVIVTFFDLKTPGRDKVLDETEYTDQEVQYKKTGGYDAPGILAAALGGQENIQADNCITRLRLVLADANKVDDDKL
KQLGALGVVHLDQNVQVIIGTKVTTVENQLEMILG

EF0146

Seq ID 427

MKKIASAGLSILVATGVAGIGGNEVQAABEQAPKTPENSSTEQPAVKATETTEQAITEKQQQVTEKQAIVDQKQVADTAK
KEKDTIDQSVKDDQAVVDQNKGALDQSQQAVTDQQAQVDEAKKVDEATPSAIEKAKNQVATDTQAVDDQKQVVDQAQADV
NQQAQAVBEKAKETNAARVQNDKDDQAVTAAKQEQAKLEELAKNAEVEKAKAEKQAAKEAELANKQKEAKAKDQKTKDD
QAVADQQTVVVTSQEKVADAKADTAARQADLTAKENALKDKQTATKQVQNTLDKSKEELKGHKGINLPANFTPDYKKLSE
QEQAMEKEALALNKVFPENQADVAKATEMINVKNPTGKQKQMSDYVVGILINDVREKLGLOKLKISNQAMRFAWDVAKYD
NPKEFDHVNAINRAAKENGPKFPGQNFYENLSMGYFETTINGTISQLEFEKAARKTIADMLFDESSAYSHIDSLLKGD
TNMAVISGDLNDISAKIHIISYNQSKLVEANTYEBGTAPVFKSKETLQKEVATNQEKLAQAQESDAQKASASQVNLN
TAKTTQATAEKELSVHKATLASLQAVATKSTTNYEKKVRQTATAEKSLOQTKDQLATINELIQNRAAVLEKARTNVSEAQA
IBQTSKVLKEKQAAQKARENTLNSLKEVLNLAKENLNQKQVAFKTSRSLSRLENAQPTYEKALNELNKAAAAVQAQEA
YENSLKSLLEELKEQAVATLAYTQAQEDLSNAKLELQQYQGVRLRELAQQAQEQQEQEALQEQVAKBQQLREBAKQSOTLV
ASATSADKTPGLQQLSFSKQKQPKAQAALTHESESRKTKQVAKAPDSLPHTEKKNKWLAIAGLIFALLGAAGIISFISRNE
KKVKNIPIKIK

EF0153

Seq ID 428

MKKMIIIALFSTSLLAGSSVSAYAQESEGNLGETTGSVLPDEPNVPTDPTPSEPEQPTPEPSTPEQPSEPSTPEPSEPS
KPTDPSLPDEPSPVPTPTPSKPEQPTPTPSVPEQPTPEPSPVEPNKPTPEPEKVPVVPVEKVPVVPQPEQPTDVVV
KPNGEIATGESSTQPTVPIETNNLSEVTHVPTVTTPIETASGEAIVAVDKGVPLTQTADGLKPIKSEYKVLPSGNVQVKS
DGRMKVLPYTGEMGIIGSLAGVCLTVLSGILYKRRKY

EF0394

Seq ID 429

VKKRLFASVLLCSLTLASAIATPSIALADNVDKKIEEKNQBISSLKAKQGDLSQVSSLEAEVSVFDESMALEKQKTLKA
KSEQLQOEITNLNQRIBKRNEAIKNQARDVQVNGQSTTMLDAVLDAVSADAI SRVQAVSTIVSANNDLMQQKEDKQAVV
DKKAENEKKVKLEATEAELETKRQDILLSKQSELNVMKASLALQSSAESKAGLEKQKAAAEAEQARLAAEQKAAAEKAK
QAAAKPAKAEVKAEPVASSSTTEAQAPASSSSATESSTQQTETTTPTSDNSATENTGSSSSSEQPVQPTTPSDNGNNGGQ
TGGGTVTPTPEPTPAPSADPTINALNLVLRQSLGLRFPVVWDAGLAASATARAQVEAGGIPNDHWSRGDEVIAIMWAPGNSV
IMAWYNETNMVTASGSGHRDWEINPGITRVGFGYSGSTIVGHSA

EF0443

Seq ID 430

MKSIKTILLGTTLAAGLGLFLGTDANAESLYTVKAGDTLSTISHQFAGDNSLIQKIASDNKLPNLDLIFEQEQLVIRSEK
VANTPAPAVEVAPVQVVEQPVAQPVQEQVQPVQAEVAQPAAPASSDAKEWIAQRESSGSYDATNGQYIGRYQLSASYL
NGDYSANQERVADQYVAGRYGSWDAKSFWLANGWY

EF0568

Seq ID 431

MKKIYQWAVGQSFKKLDPQQVKNFVVFVYLGALITTLICFYPMGIFLWFNISITIFLWLTLFANFAEVAEGRCKAQA
DSLKQAKKEVMTYKINSLEDIKEENFIELQSSDLKRNDLVYVRAGEQIPADGDVIEGAASVDESATGESAPVIRESGGDR
SAVTGGTTVVSDYLVRVTSENGQSFLDKMIAMVEGTQRKKTPEIGLQIFLITLTIIFLTVSITLVFPFTDF88QLSGKGB
ALSIVIALLLICLAPTTIGALISSIGIAGMSRLTKENVLMSGRAIEAAGDVDVLLLDXTGTITLGNRRASDFLPVHGVS
EQLADAAQLSSLADETAEGRSIVILAKERFNLREREFQSEVKFIDFSAKTRMSGIDYRGDVIRKGAADTMKKYVQSKGE
DYPSECDKIVDKIARAGGTPLVVVKNNRVNGVVYKDIIVKNGVKEKPADMRKMGIKTIMITGDNPLTAAALAAEAGVDDFL
AEATPENKMNLRBYQEKGHLVAMTGDGTNDAPALAAQADVAMAMNTGTQAAREAGNMIDLDSSPTKLLQVVQIGKQLLMTRE

GALTTFSIANDIAKYFAVIVPVLFYSIYPQLDRNLIMGLSPLTAILSAVIYNAVVI VALIPLALKGVRYQEKPASQILSHN
LLIYGLGGIIPFFIFIKIIDLILSLIIL

EF0591

Seq ID 432

MFFTGKERNLKFALKKHGGGGAASVIIGCIIFGSIAPGGTTLAYADEVHNSINQDIQDSGSGTIIGENDSSTKSAEYKMIH
EIDGTKISNGENSKETTTSSGTILAEZAISSNQKNSKTSFEVQDLHKDVSGSESVKQVETSDSIKKSESAVKTLNLDDSD
QENTNSITTKAENDALSTVNDKVLNEDSIIKSIPTENVDNNSKSDNRDLVENSVEVTKKIMREADKTEIAKRNDNIS
VLNAGTESFNDSDKIRRSKRDNEPVPVSLSIQMPVTKEIYENDSTIKGKSEPGVTIKIYKNGIELGSIKADDDGNFEYPLB
SQAKSDNYSFVAEKDGAKSIESSVNVVENKEIVGNQRDAYTPYGFYINRGLVTTDDGKXHYTVDMEGNLASEAYFDLAG
GTMYSIDEKFPAPYVEKIVLDGKTIISKNFYELPNKNTIWNSSGILSTDKRGLVRAALVGRVTNGTIDITYFKDDTPQEVLS
EIPFQVWARFKEKKGKNDLISDFDLNILLQNNKLILSKDTFFKDEDIVNNNGPKISSNFNTNTIDINYGVTNGNYGL
TIGKRSYPYLNHINTGKELSDLVDTIKIDNKNYBEKLPDGSVLKDIYKKGILGGALLNRQBITTSLGLIAKKNLGDLDIF
KQELLELDVSIIRDNTFKLVKGEATEARKIDSSFDTFKQELTKWIERDSDNPQEPITINSQDNTSIVDPKEMVEFAVKNP
TTENINKARSLVSKMVLWENKYNPVLNAAEEVNRYLSTTLNGSSNDGLILNNTAAATFVKLIDTDKDGILDRYANMGI
GTINYTNADSDGDKSDGFEIFNDTDLVSPYDWFDKNGEKIKIVTTDTTISGRIGNNNYDTENVYPRVTQLIKVTDNEN
LISEISSSEDRKGTPEFTGLSGKLSKGRDLVVKIITNEVQREIDRQRNVVQVGYDNPEVSEBEVIVQGAQVTVSFDENYNT
DPDFDRQPTVQVVELEKGRLLNSYFTPERKGYKFIGWNTDLGVGQTVNDGSSNFNEDITVFAQWEKEPNLAGEVHAPQGP
IEEGKSIVLNNVQSNKSGSVIVTGLPKGLFGLSINETGNLIGTPLINDWIDGNSREVKIPVTISNGDEKVMVEVPLTI
LHDTRAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDTTAKKEDASKLVDALEPGPVKDSLKDRLDKVTT
EVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDTTAKKEDASKLVDALE
GPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDT
TTAKKEDASKLVDALEPGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTD
GLVTPPEKAAVDGLNDTTAKKEDASKLVDALEPGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAA
EDAGKAGADKKAETVTDGLVTPPEKAAVMA

EF0592

Seq ID 433

LPPEGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGL
NDTTAKKEDASKLVDALEPGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAET
ETDGLVTPPEKAAVDGLNDTTAKKEDASKLVDALEPGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAV
KAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDTTAKKEDASKLVDALEPGPVKDSLKDRLDKVTTSEVTVDADSN
GKADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDTTAKKEDASKLVDALEPGPVKDSLKDRL
DKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGLNDTTAKKEDASKLV
DALEPGPVKDSLKDRLDKVTTSEVTVDADSNKAGADDVLAEKAAADAVKAAEDAGKAGADKKAETVTDGLVTPPEKAAVDGL
LLEIKQSSFPMPFEN

EF0658

Seq ID 434

MKRKINYTHIAEPTVFENVPFYLNETMARKQKVLQRMETEKFDQLVFYADKEHGSNFEYLTGFIIPRFEGLLVNLKDG
AATLILGNENLKLQCHARISADLIHYLAFSLPNQPMAGEQKLSQIFETLLDETAQKIGIVGWKMFTTQQQEPATMFDVPHF
IVEALKRALPKPEARLLNGTHLLIGAKGARATNNANELAHYFYGANLASRMLKALNAIEIGQRETDIGALLNDEGQTPVTV
TIAATGQRFYANMYP TAKEIQLGDALSLTTGYKGLSSRTGFVINEQQLPBAQRDYLERVAKPYFQAVVHWLETIRIGL
LGRMYQAIKEQLPKIYHWHLPNPHLVSDDEWMSPIYDPSAIRLESGLMFQVDIIPSVPGYTGVSABECVALADETLQK
EQQTYPDMQWRATRAKYLKETLKLDPSEVLPMNLVGYLRPFYLAQKALCVKEPKALK

EF0727

Seq ID 435

MKKARVIYNFTSGKELIKKNLADILSILERCGYEASAFATTPPEENSARNEAHRAARAGFDLLVAAGDGTINEVVNGIAPL
KRPRKMAIIPAGTTNDYARALKIPRDNIVKAAEVIKKNQTVKMDIGQAGKNYFINIAAGGHLTELTYEVPSELKSIIFYLA
YLAAGAEMLPRVPIKMRNTYDEGVYEGNASMPFLGLTNSVGGFEQIVPDAKLDDGKPSLIIVKTANIFBIHLVALMLNG
GKHVEDHRLIYTKTSYLHAETLEKNKMMINLDGEYGGDAPMTFKNMHQHIEIFANGDALPSNAIMGSVLTSDEIVVESE
DREERAYNEASKEFVKEVERLTDDEDIDGDKIARKEKH

EF0775

Seq ID 436

MTNTVKVKDDSLADCKRILEGQATFPVQAGETEPVDLVVVEDASGSFSDNFPHVRQAIDEVVQGLSDQDRVMLASYRGGKQ
FMFPDGGTKINSADYDMNVRVNTQLTYDKSQFVSGFGDVRTYGGTPTAPGLKALDITYNQTHGDLTNRKTYFLLVTDGVAN
TRLDGYLHKTNTNDSINEYDPDRHPLQVSVEYSNDYQGAEEVLALNQBITNQGYEMINAYWESVESLSSVNSYFDKYKTE
VGPFFVQELQGSSTPEDFITSSQSIDDFTTQLKQIVKDRLAQSTPATASLTIANQFDIQSATATDDAGNDVPVQINGQTI
ATSTEGYVGNITTHYEVKENTAIDAATLVSSGTMTNQGTIAKEFPPEATIPKNDNAHACDVTPEPTITKDIENQEHLDLTNR
EDSFDWHVKTAFGNETSTWTQASMVDDINKVLDIITDVKVTDENGKDVDTANGVTQENNKVTFEMNKQADSVDYLSGHTYTM
TITTKIKTADATDEELAPYIEQGGIPNQADLNFNGEGDVLHNSNKPTVTPPPVDENIADVEGQEHLDLTNRDQEFKNVKT
AFNETSTWTQASMVDDINKVLDIITDVKVTDENGKDVDTANGVTQENNKVTFEMNKQADSVDYLSGHTYTMITTKIKASAT
DEELAPYIEQGGIPNQADLNFNGEGDVLHNSNKPTVTPPAPTPEPTITKDIENQEHLDLTNRDQEFKNVKTAFNETSTW
TQASMVDDINKVLDIITDVKVTDENGKDVDTANGVTQENNKVTFEMNKQADSVDYLSGHTYTMITTKIKTADATDEELAPYI
EQGGIPNQADLNFNGEGDVLHNSNKPTVTPPAPTPEPTITKDIENQEHLDLTNRDQEFKNVKTAFNETSTW
TVNPLYMIAGLIVLIVAIISFGITKNKRN

EF0779

Seq ID 437

MSAWQTFKNGTRQPFKDILQYLWLFFTLNVLVLLVGGAFSWATSNALKTQGIPIYLSFNNLNLLLEKPLALVLLTILLLLLFL
GAVFYQFTFLLLGIFQIRQDHRFHFKGVTKASFVKVLKKQGARSWLFFFGYFVVI VPFNGNLIQSNLLTKFVIPDFIVEFLS
QRIPYLVGLLAFGLLVWYLAI RFIYTLPLMLERKKAGEAVKASWSMTNKRLLWFIIRNIAFVTIAVVFSTYVIYVLLYLLQ
LKLDTLSDTISLLGGILNLTVVQFLQFVSNANLSVLLINFLYTQLNVQAETTTKVAFDKETKRNKLVTIGMGLGLFTIFGG
YIIFNAVYLTGLLESEPLIISHRGVTNSNGVQNTI PAMERTIKFKPDYIEIDVQETKDHQFVVMHDANLQELAGVDGTPQE
FTLAELTKMTVKENGQEAFLASFDYLAQANQAKQLLVBKTSKQDSQGALSNTFKYERPLIKNNHQVQSLDYNVIKAF
KKAISKVVSFILPYNFTFETQADLYTMEATTINDTFILKADQKKAVYAWTVNDSEVLSEMLFMDVAGVITDDLELVNE
EVNDFEKNPSYADRILHYIFMLPSVASQ

EF1091

Seq ID 438

MITDENDKTNINIELNLLNQTEQPLQREIQLKNAQFMDTAVIEKDGYSYQVTNGTLYLTLDQAQVKPVQLSLAVEQSSSLQT
AOPPCLLYENNEYDVSVTSEKITVEDSAKESTEPEKITVPENTKKTNKNDSAPKTEQPTATEEVNPPFAEARMAPATLRA
NLALPLIAPQYTTDNSGYPTANWQPTGNQNVLNHQNKDGSQAQWDGQTSWNGDPTNRTNSYIEVGGTGQADYAIRKYAR
ETTTPLGLFDVYLVNRGNVQKEITPLDLVLVVDWSGSMNENNRIGEYQKGVNRFVDTLADSGITNNINMGYVGYSSDGYNNN
AIQMGPFDDTANWQPTNPIKNITPSSSTRGGFTQKALRDAGDMLATPNGHKKVIVLLTDGVPFTFSYKVS RVQTEADGRFYGTQFTN
RQDQPGSTSYISGSYNAPDQNNINKRINSTFIATIGEAMVLKQRGIEIHGLGIQLQSDPRANLSKQQVEDKMRMVSADEN
GDLYESADYAPDISDYLAQKAVQISGTVVNGKVVDPIAEPPFKYEPNTLSMKS SVGPVQVQTLPEVSLTGATINSNEIYLK
GQBIQIHYQVRIQTESENFKPDPFWMNGRRTTQPLATAPEKVDGVPVSGKAPGVKLVNKKIWEZYDQDPTSRPDNVIYEI
SRKQVTDANWQPTGYIKLSKPENDTNSWERKNVTQLSKTADSEYQEVGLGPQYNNQGOAFNYQTRELA VPGYSQEKIDD
TTWKNTKQFKPLDLKVIKNSSSGEKNLVGAVFELSGKNVQTLVDNKGYSYSLPKDVRLQKGERYTLTEVKAPAGHELKKK
TTWQIEVSEQKQVSDGQEVTTTNTQVIPLBIENKFSSLPPIRIRKYTMQNGKQVNLABATFALQRKNAQGSYQTVATOKTDT
TGLSYFKIISBPGEYRMVEQSGPLGYDTLAGNVEFTVDKYGKIHYAGNIEENAPEWTLTHQNNLKPFDLTVNKKADNQTL
KGAKFRLTGPDTDIELPKDGKETDTFVFENLPGKYVLTETFTPEGYQGLKEPIELIIREDGSVTIDGEKVADVLLISGEKN
NQITLDVTNQAQVPLPETGGIGRLWFLYLAISTFVIAGVYLFIRRPEGSV

EF1323

Seq ID 439

MGILLPIIIAIVLVLLMLLIVFVSKYQTAKPDEALIIISGYSYLGSKNVHVEDEGKNKIKIVRGGGAFVLPVFORSNRISLLSSK
LDVSTPEVYTEQGVPMCDGTSIIKIGSSVEEIIATAAEQFLGKTTRELENEAREVLEGLHRSILGSMTVBEIYQNRDFSQS
VQEVASVDLAKMGLIVIVSFTIKEVRDKNGYLDLSLGPRIAQVKRDADIAEABALKETRIKABAEKESQQAELQRQTEIAE
ASKEKELKLALYKQEQDIAKAKADQAYNLESARAQQHVVEQEMEYKVVVERQKQIELEKEITRREKQYDSEVKKKADADRY
ARQBALAQKAREVABAEAEERFKVEALAEABANKTRLTGQAQABAILARGAAEABAKQKIADAFKEYGEAAVLSMVMEMLP
QLMKEAAQPLGNIDKISVVDTGAGGENSGANRITNYATNLLAGTQETLKETTTGLDVKELIENFSSKGTNSVNYHATEGSE
KE

EF1355

Seq ID 440

MAYQFKLPDIDEGEIAEGEIVKWFKVPGDTINEDDTLLEVQNDKSVVEIPSPVTGTVKNI VVPEGTVANVGDLVIEIDAPGH
EDNDAAPAAPAEQTPAQPAAPVPTTEAAGGFFQFKLPDIDEGEIAEGEIVKWFKVAGDTINEDDSILEVQNDKSVVEIPSPV
TGTVKNI VVPEGTVANVGDLVIEIDAPGHNSAAPSVAAPATDAPKAEASAPAAS TGVVAAADPNKRVLAMP SVRQYAREKD
VDITQVTA TKGKGRVIAKADIDAFVSGGSAAPATEAAATEAAPKAEAAAPKAAPKFTSDLGEMETREKMTPTKAIKAM
VNSKHTAPHVTLHDEVEVSKLWDHRKKFKDVAAANGTKLTFLPYVVKALTS TVQKFPILNASIDDAQAEIVYKNYFNIGIA
TDTDHGLVVPNVKNANTKSMFAIADETNEKAALAI EGKLTQAQMDRGTITISNIGSVGGGWFTFVINYPEVAILGVGTIAQ
EPVNVADGEIVVGRMMKLSLSFDHRIVDGATAQKAMNNIKRLADPELLMEG

EF1699

Seq ID 441

MEYTIKKMASLSGVSARTLRYDDEIGLLQPARINSSGYRIYQAEVNRLQQILFYRELDLKLDEIKIELEQPDFNVEQALY
EHQQKLEKRENEIDRLLASVQQLLHHYKGEINMSDQKFEAFKQKQVQENBEKYKKEIREKYGNETIEQANKYLNLTEDK
MQAMQNVKDLFSKLAMYQKSPKLTSQLAQBI FQLHEDWLMYSWSSYSPEAHKGLGLMYGDERFTSYE QHGAGFAEALN
AIIQNYA

EF1744

Seq ID 442

MAKKGFFLGA VIGGTAAAVAALLAPKSGKELRDDLSNQTDLLKNAQDYTDYAVQKGTETELIAKQKAGVLSDOASDLA
GSVKEKTKDSDLKAQGVSGDMLDNFKQTGDLSQPKKAADDAQDHAEDLGEIAEDAED IYIDVKDSAAA AKETVSAGVD
EAKETTKDVPKEAARAKEDVKDAADVKKEFKG

EF1752

Seq ID 443

MKRKLTKSPNNVLTGSLAGIADWLGDPTIIRVVYVLLSFFSAGFPGLLYLALAVLIPSGRTGSDRGYGHQNPYNRNR
NENPYAANKQRKBAKIDDEWSDF

EF1753

Seq ID 444

MKERERVLELVKKGILTS EALJLLENMATEKDEKQIEKAAEKVDTONIGTTNKEDQVADLMMALEKGESEGTVDSPFEN
TQDSAEKDRENLERILDELATKANRASAE LDEVNAE IAGIEEIKEVAEEIGTLDTKESLDALTEDEQVQRKDLHVLLAQL
EEKLATQSTETKALEEELKNIRKEQWKQWQNDTKEKVSSQFSEEWKQDQATDTFNQVGGKVAE VGGQVGEFLKKTNSFSDT
MNDNVEWKDIKMKVPGVATTKPEHEBFNYPNPQASLIDVKVANGTVVFETWDQEDVKVEAKIKLYGKMAGDSPMEAFLESD
IDVQDLETISFQVFNKRKADLTFFLPKRTYDEVS VKLLNGNVLVEELTAKDVYTKSTNGTITTFKKIDATMLEIBGVNGEIK

VLEGTILDNIIFTVNGDVSSISAAPESLSVSLINGDIRITAKEKTLRRVEASSANGNIKLLALPNDLGVEGVKTNLGSINSR
LTDIEVVREKKDRGNQQLHFRRVLEESMAQINASTTTGSIPLKDTDK

EF1791

Seq ID 445

MKKYLKIMVCIILVGLAGCTINKNENKQKNTKEAVQLMSPSELTTLNLSVLLDFPDAIVQTAAPFEGLYSLDEQDQLVP
AVAKALPMISEDGKTYTISLRKEAVWSNDDPVTADHDFYAWKKMIDPKNGFVYSFLIVETIQNGAEISAGKLAPNELGVTA
VDDYTLKVLKEPKPYFTSLLAFPTFFPQNKVVEQPGADYGTASDKVVYNGPFVVKDWQQTCKMDWQLAKNNRYWDHQNVR
SDIINYTVIKETSTALNLFEDGQLDVATLSGLAQONKNNTLYHSYPTATMNYLRLNQKRGQATPLANENLRKALALGID
KENLVNNIADGSKALEGAIIEGTFVANPTTGLDFRQAGNLMVYNKEKAQSYWKAQAELGEKVNVELMVTDDGSYKKGIG
SLQSSLOQLFPGLTIKLTALPTEAALNFGRESYDYLFLIYWTDPDYQDPISITLMTLYKGNDRNYQNPVYDKLLDRAATTYAL
EPEKRWATLIAAEKEVITETAGMIPLSQNEQTVLQNDKVKGLNFHTFGAPLTLKNVYKEK

EF1800

Seq ID 446

MKHGKIKRFTSTLLASATILVPLSTABETTNSSTETSSSMVEPTATEEKLWQSDFPGGKTGEWQDVIGKTNRELAGESL
AISRDAAAGNNVSLNLDSPKLADGEVETKFKYTAGSGRTGVIIRGNTKDSWVFGYNANGKWLVBSPNSWNDSISGPTLN
EDTNYLLKVRVYGEKITIWLNTLIYEGBFVLANGDKIPTAGHVGLVRLWYDKKIVNYDYFKNGFVDSIPEIVEPTQLAP
VKVFTKIGVAPKLPKQKVVTYNTGKEANEAVRWNEIDPDAYKEPGTFEVDGTLNNTKAKASIVVAKDNEAEKGDKISSA
DLTAVVDPPQFPRIIRYEDPQSNQVIFNGQHEKIDQVMIDGKAYKATAEKQKSEANQAVYNVAVPEIGLRFPTTLTVSEGOE
LAMKLSDIRREBTKIHTISIPNOGLISVNSTDEGATFAGVVMNTGTNANNNGNKGDTIQDLTTTSQESTKKMYGFLNTAN
YAASFWTNAYGDSGSDNNRIHKQTEAATGFVTTLSSGAWTYRPFDAPEYTTGETPEVKVKFSKDSNDDNRVDWQDA
AIGERSIMNPMGAEKVPELVNQRIIPNFASQATNPFLVTLDESKRIYNLTDLGQMNLLKGYQNEGHDSAPPDYGAIGQR
PGGEQALNQLIDEGHKLNAVFGVHINDTESYPEAKGFNEELVDPTKRGWDWLDPSYPIKQRPDTLSGRYERFKELKQKAP
NLDYIYVDVWNGQGESGASRQLSKEINSLGWFTTNEFFNALEYDSVWNHWSAEKDYGGTTTKGFNSTIVRFINHQKDTW
IISDNPLLGGAEEFAYEGWVGKTNFTNTYRQKTFAINVPTKFLQHYQITNWETTTAADGQIYGTIKLANGAEKVTVTQADAN
SPRSITLNETEVLKGDAYLLPWNVNGQDKLVHWNPKGGTSTWSLDKMKQKTNLHLYELTDQGRIDKGAIAATTNNQVTIOA
EANTPYVLAEPDSIEPMTFGTGTFFKDPGFNEANTLKNWVFRGDGEVKKDANGDYVFSSEKERTETIKQDINLPKPKGYS
LYLNTETEDRKATVTYKLGKKYTRTVANSVAQNYIQADINHTSRKNPQYMNMRIDFEIPDNACKGSVTLAVDKGNSVTK
FDDLRIVERQTDIMNPDKQTVIKQDFEDTQAVGLYPFVKGSAGGVEDPRIHLSENEPYTYQYWGNGNLVSDVLEGNWSILKA
HKQAGALMLQTIIPQNIKFPNKKYTVQFDYQTDGENVFTAGTINGELKNNNDFKPVGELTSTAADGQTKHYEABEIGDASG
NTTFGIFTTGADKDFIMDNFTVTVESKK

EF1818

Seq ID 447

LMKGNKILYILGTGIFVGSCLFSSSLFVAABEQVYSESEVSTVLSKLEKAISEAAAEQYTVVDRKEDAWGMKHLKLEKQT
EGVTVDSDNVIIHLDRNGAVTSVTGNFVDQVVKIQSVDAIGERGKIIASDNPETKDLVFLAIDKRVNNEGQLFYKVRVT
SSPTGDPVSLVYKVNATDGTIMEKQDLTEHVGSEVTLKNSPQVAFNVPVKSNTGIALHGTDNNGVYEAUVGDKNNYSIIQ
APSLVALNQNAVDAYTHGKFVKTYEEDHFORHSIDDRGMPILSVVDEQHPDAYDNAFWDGKAMRYGETSTPTGKTYASSLD
VVGHEMTHGVTEHTAGLEYLQSGSALNESYSDLMGYIISGASNPBEGADTQSVDRKTGIRNLQTPSKHGQPETMAQYDDRA
RYKGTPTYDQGGVHYNSGIINRIGYTTIQNLGIEKAQTLFYSSSLVNYLTFAQPSDARDAMLAALKVQYQDEAASVVSAAF
NSAGIGAKEDIQVNPSESVLVNE

EF1850

Seq ID 448

VQQNNNEISTQHOFDAYCKKVLNRNEAKSIRKRNDEKIKENEPLNDLNEGKYSQTHLDEQDVYFLFGMEILLISDQKLSAAID
QLSDTRKIVLLYYFAGFNDTBIGKIFNMSTSGIYQRTKAVEBQLKMEYGLW

EF1877

Seq ID 449

MKKKLFRIVGLVFIISILTLIVLLSLVGTVAEATGLVDDTVESGNLYSKYSLNNYQLDFFVDSWDWLPWNWGDGLGKSVMY
GLYAITNFIWTVSLYLSNATGYVVOEAYKLDFISDTABSIGKNQITLADITENGLQTSGFYFGFLLMILALGVVYAYTGL
LKRETTKAVRAVINFMIFLLSGSF LAYAPTYITKINDFSSDVSEAAALTGTRIVVPNSSESQKDSVDLIRDLSFSIQVQQ
PWLLQLQFDDSNIEEIGEDRVNKILSVSPDENKGDREARAVKAEIEDNDNANLSITKTMNRLGIVVFLVLFNIGISFPVFL
TGIMLFSQILFIIFAMFLPISFLLSMLPTYESLGKMAIIRLPNTIMMRAGVTLVITTAFSISTMFFNISATYPPFMVAFLO
IVTFAGIYFKLGDIMSFMNLQSNDSQSNGRRVMRRPQMLMNRKLRQLNRNVGRTLAFGAAAVGNKLAKEQSKPKFKPAGS
SLRKNRSLPNDREVSSDSAKENPISNNKKQSRMNLTKRKIGKVLDTQALVKDKAKQVKDQVRNTPTNLKYILHKGLEKTRK
APKBFKRGVLQEKADREKLDRKQRRDEKMDKRNKLGVTDRHKKRRSNVSIKEDPKPKQDRILKNELPKRIVMVKPNS
EVRRLVQKQNLVARENNQLLSKKNSPQVQQRSTLPKRANQKVQRIKARPKPKSGDKK

EF2174

Seq ID 450

MKKIISGMLICTVLLNSPVSIVASGELVKTETTTGETGLVTQATSETTTNSTEDTSSVTEENTSERDSSTSSSTKEESLDSST
NSTTEHSSSVSETNTIDSKTEASQSSEVEKKTIDQDEADYQEAAREGTHKKGTYAMKNGLSSRVARATVANVYANDPNLP
GKNFIDVSSWNGDISVAEYQIKSYGVTGVSVKLTEGTWYVNPYAAAGQIRNAKAAGLKVSAHYHSMYVSAATAQDEARYFA
QAAANSGLDKNTIMPNDABPTLTNNGRNAHANSVAFNQKALKALGYKNDALYVGKWWLTNGYIDTSAPGRDRVWVAQYPYT
PDSSMQWNNDEGAQWSSQMYFPGLANYEGRPPDISMTYSNFMNMGNSGGPDLSSKYTTNPRGRVIMKNDTFFYQVAFRTP
GWRVKKNTLVITIKGIEYSSAGIPRLVTDQGYLTANKDYLAAQSNIDLYFTTNPKKVLKSDDYFYADPBFKQRLSKVSKG
TIVEVEDLAYTQSGIFRLKTAKGYLTANKNIVEQYKGLDITYTENPGQVITRNEDTYKDVFEFKTANKVSAAGSVLKVTA
IEKTSKGIPLKLMANGYYFTANKNYVVAATGSWIANHYTVNPGQIIMKNSDNFYGDSDFLYKGAAVSKGSLVPVVGIEYREN
QVPRLITQNGYLTANKSYAQKVVPNIKDYLYDYPEYVMKNTNDYQVNFSSKKGFEVSKDTLTKVLSVDYTENGLPRLEK

Seq ID 451

MNKA VKNFVS YL MITMLF ILNL LPMN AFAQEV TS DA EKTV EK DGLK VIGK IEDT SSQ EDIKTVY EVNTRD VPIK D LIL
KQKNTNDSP I K FVLD T L S EERGPT SLEBQAKVETNEKDQD TDIK LNLQPNSTRK I TNGQITTKASNKLLVSVLIEDNEK
GTLVIDLPSKD ILADKESVSKEQKETS T ETKVQNANFTASSTNEMTATTSNETKPEAGKAIES I QETALTQATESPEQPFPL
KAQPTGLVPPTPGRGFTPIYQSVHKGELFSTGNTNLKIANENTAAQGTFLNTRGASSGYA I NFPLEFADVDNDPNTYN
SSRAYIDLNGAKELAWAGLFWASASRYKGPAYGTNLSD EISAPVQFTT PNGTVQVRVSPQRYHRIDQDATNPGQRFVGYNTG
FSNYADVTS ILQGDKSATGSGYTLADI PMTSSSLNGQYQYVNSGWSL FVVTIQDQASKSRAPS IYYGARGNAAGT NNEFTMSN
FLTAKQGNLDP I VTWSTVQGDKYWTGDIGMNAQIKNSAGTYWVNI SNTLNPVNMMATNVTSDND EHMVDKPGKPA DPHNPLDI
DIDRMAI PEGVLNAGONQ INFRITSSGDYNTS AIGFVNAETPEFEI KEI VBP E KTVYKVG ETTIYRVSLKNTKADSEAI
NSVSKDALDGRNLNYPGLSKIIISGPNSEKGT DASGDDQABYDET NQIIIVRVGNQATATQGGSYKADTAETIYEFKARINE
RAKANELVPNSATVBRVADILTS AKVNETSNI VBAKLADEQVTGLKATATVNNAKVPLGEEIETTISFRNTIENGILNKVV
ITDQLPKGLTYVDLSLTVGDEPKPTS LKETNGTITAEYPS ITDMKERSIRFKVIVNEEAKAGETILNKA KVDVTNPPPE
PEVPVVPKTNAGKLAATKT VNNNAKPLKETIETTISFRNTIENGILNKVVITDQLPKGLTYVDLSLTVGDEPKPTS LKEI
NGTITAEYPS ITDTKERSIRFKVIVNEEAKAGETILNKA KVDVTNPPPEPEVPVVPKTNVGLKTATKT VNNNAKPLGEEI
EYTTISFRNTIENGVLNKVVITDQLPKGLTYVDLSLTVGDEPKPTS LKEINGTITAEYPS ITDTKERSIRFKVIVNEEAKA
GETILNKA KVDVTNPPPEPEVPVVPKAGKGLTATKT VNNNAKPLGAEIETTISFRNTIENGVLNKVVITDQLPKGLTYV
KDSLTSVGD EPKPTS LTEANGTITAEYPS ITDMKERSIRFKVIVNEEAKAGETILNKA KVDVTNPPPEPEVPVVPKAG
KLAATKT VNNNAKPLKETIETTISFRNTIENGVLNKVVITDQLPKGLTYVDLSLTVGDEPKPTS LKEANGTITAEYPS IT
DTKERSIRFKVIVNEEAKAGETILNKA KVG DGINPPEEPEVPI TPEEPAKNKKEITNKVVTDQNKPTKNSKNEI AINKKETS
KSSYL PKTGEKVQKI FAXLGVGLILILVILVILVIRKNEKEE

Seq ID 452

VSRRFQAREKKVQXMGDRGLVEQNRATGEEKRVSQRTADTAFDRAPRTEREDIRKPAARGADTGKKRQFRTAFEMAEQ
TEPLLTPEYLLADSEPPPDAPASMRGAVDMPILLDAPLABEPTPAPVRKQPKRGKGGKKKQSTKFTEDAARPGSEKKTAM
RGVEGAPAPKRTSEGGSKRLQFDKEEPPADPADRAAIRKQTKAKLAHAAPKEDKLKSDGNPKAGETRLKFEFDTPKDITD
SGDTAPDMPTRQKKYKNAACRTERADRRVEQAQMKLPAKRLRHMDHQPRASGKIKKRLRFEDEILPEYQKPSLSSRAGN
AAKTAAVLKLHGKLEREYERDNVALESAHKIELVAEQGAGRVLRWBNRRRSKPYTRLRAQQKAAHAHTDLAWQATLRDNP
ELQRQNALSNVYQKQIKKKYQAQAHEAKYQKPTQNVLTATGKIAARAIQYAAAHKAVFLAVAMLALVVMFFATGLTSC
TAMLSGFQSSYISAWYMANEKEIQSDLYSTKETDQLIDIKTEENTPGYDEYRYNIGIBISHNPVELLYGLYSLTAFNAFTFA
EVQPEIDRIFSRQYTLTREVITVEITRYDDGDPYDWYQLQTLAVRPLSSVLQSLTPEGTDRYGVYMGTYGNRQAFGNPF
GFSWLGYVSSGGYGRVHPVNGEKSILHRGIDLAVAQGTPIILAAQDGRVVSAGDAGSYGLCVVIEDKGYQSYAHCSLNLVS
AGQSVKRGRDVIAAVSGSTGNSGPHLLHVBMLNGEYILNPYFFVDNGDDGTGAIPTGTPGGPAIPDYSGBPMGDGSEAMLR
EAYLGYPPYVWGGHSPSTGFCSSGYVSWVNLQSGVSGVGRQTAQGLYNLCTFPVSAVNAQPGDLIFFTGTYSSPGFVSHVGIY
VGGGRFIHCGDPISYANTGSPYVSAHLYGYGRIP

Seq ID 453

MTKPEWEAWS PAKVSSQEDFLAWYEREKRNLPWRANTDAYIRIWISEIMLQOTRVDTVIDYFYRFMEWFPTIQDLARAPD
 DKLLKAWEGELGYISRARNLKVAQQIVSEFGGKMPDTIEDIRSLKGIGPYTAGAIGSIAFNLRPAIDGNVMRVVSRLEFET
 DADIAKASSRKVFEAAMLKI IDRRPGRDFNQALMDLGSAVCTPTSPKCESPLQYQCAAYQADKMTAYPVKSKVKPKDVOY
 VVGTI IENKKQEFLLERQFETGLANMLWFLIEBISKQFQQLQKLAQPAETEKQLTLELEPMTLEAEPEPNVFFTTDYET
 VVWQKRTLGEVVHIFSHLKWHLVFGYGRNTGELATLLESQRWVQKQSDYVFFPKPOKMVPLFKEHEEK

Seq ID 454

VEKSTVTSLTQETSATTINASTDSTALTAESERLPSLRQTLINLYVGMYGLTETLINRLSDDELIDYAKKVSFHFVNQDISGT
ARMITKLYGEKPIPEDSYSTDYSTLTIDDLKNYLPQIRLSLIYVYDLNSDVVNNLSQDTLVDLINQVKVDYANQNPSPDVR
GDYGLAAMADKIKANDYTSINQSAESVSDTTNTSTLTQTTSSKKATTSSSTEKKKGIFPSTGEKKSVLFTIIGIILLS
LVSIFPIKNKKK

Seq ID 455

MKKKILVGLVALVFFMPLNVFAAKGDQGVDAIYQGEQGRFGYAHDKFAIAQIGGYNASGIYEYQTYTKTQVASAIAQGKRA
 HTYIWDYTWGNMDIARTMDYFLPRIQTPKNSI VALDFEHGALASVPDGGYGVYSDASEKAANTETILYGGRIKQAGYTP
 MYSYKFPFTLNLHVNYQQLIKEFPEKSNLWIAAYPIDGVSPYLYAYFPMDSGIGIQWFTSAYIAGGLDGNVDMRTITDGSYTD
 TNKPEPTDTPATDAGEEIEKIPNSDVKVGDTVVKVKSVDANATGRAIPDWGWKNSYKVQBATGRSVLLBGILSWISKGDIEL
 LPDATVWVPDKQFETHIYQHGFTLSAIAYQYGTDYQTLASLNLANPNLIYQGVQLKVNGSATSNVYTVQLGDNLSSIAAK
 LGTTYQTLLAALNELANPNLIYQGTOTLV -

Seq ID 456

VSQKQSDVVLNFKMNGEINYSRITIKDINKEMNLAAATYKNQVSAMDKNATQTEKLATATKKLKEQLSLAEQRTKLLREYYE
KSVKETGEYSEQSQKLYKRLLESSETGENKLRSLALQSTNEALKEQGNLSIKTABKLAKIEKAGDKIKSVGQKLSVGLTAPIM
GIGAASIAAFKELDECLDNITTATGATGSQLESLSQASFKTVAGQIPADMQDISTGIGEVNTQFGLMDKQLEDTTGRMLKFS
EINGSVDSQSTINAKKSMDLFRLSIEDLPMILDSVSKTSQDTGVGVQDLFDAVNRGAPOLKAMGLGFSSTTLIGOMEKAG

IDSAGTLGYLAKASVVYAKDNKTMQEGLSGTIESIKGATTEQEKLTIASEVPGTKAASKMVEAIDSGALSMDGLADSAKNA
AGTVDTQTFSDILDPIQARLAQNQFKIAMGELGEQVQIALLPAFQAATDAIKKVSEWFGSLTDSQKQITLKIAGVVAAGP
VLVVLGTLASSISSLIPIVIAPIASPIGLVIAVAWAAIVVAYNKIGWFRDFINTSPKVIKDIVVGVFNVLKDTTKSTFD
FITGFIGGANDGAAKIIGDYVNEIKRIFGGIVDFVTGVTGDWSRAWQGVVDIFGGIFEGIAAVAKAPINAMITLINGFIG
GLNNIKIPKWPVPGIGGKGFHIGKIPYLAEGGTILNGQAIIVGEAGPKLLTAKNGKTTVTPLSPEEKARGIGGALKGKNTIEQ
HVHIGQVDANNPSELDRMNRKLYKASQAQAFYDLGGVPT

EF2820

Seq ID 457

MNKKLLRQLQARHEKRLSDLQKIESGEVREADLDSVNEEIDGLIDELKAIKAEKGDDNSESQDGEQDDGTAKSDNTDDES
KEDREKDTNGNNDKNEENRGGMISQEQRDGLLRTIHEGMEARNVMSNEQREKQIRKAFADPVGINISSEARALGIETGN
GSVTVPEVLAASEVISYAQENLLRKYGTVIRTAGDVKYPILVKKARANVNKKERTTDTITTAIQFDEILLDPABFDALATV
TKKLLKMSGVPVEDIVVEELKKAYVRKEINYMFGDDAGNENPGALAKKAVAFKFPVDLTAAGAGQKLYDALIEFKNTPTVT
EVMKKGRPIINRAALTAEKMKTTDDGFPLLRPFTAEGGIGYQLVGYVPDWTDAADKKGEFDTFVLYFGDFSAPKIQEVIG
ALBIQKLVKFKSGKNQVGFQIYNLLDGQLVYSPFEPVAVRYEITKPVGG

EF3082

Seq ID 458

MKKKFLAMMAVSMGLMLLSACQTNKKTADSATTETTAKTEVTVKDTNGQLTVPKNPKKVVVFDNGSLDTMDALGVGDRVV
GAPTNIIPAYLKKYQKVESAGGIKEPDLEKINQLKPDIIISGRQODYQEQKALAPTIYLAVDKPNWASTKQNIETLGT
IFDKBEVAKKITGLEKEBIADVKKQAEASANNALVVLVNEGQLSAYGKGSRFGLIHDTPGFKAADDKIRASTHGQSVSYEY
VLEKNPGLFVVDRTKAIGGDDSKDNVAANELIQKTDAKNDKVMIMLQPDVWYLSGGGLESMLMIEDVKKKGLE

EF3256

Seq ID 459

MKVNFVKGFAAIALSSLVLAACGADKKDNTTNSSSAASSETTKSTESSAPAKKVAGGDLKDGTYKLEEKNEKNGYRAVFE
MTVNDGKITTESKYDNINADGKSKTETDKYEREMKAKSGVGPKYIKQLNDSFVKAQASGVEVVTGATHSSSESFQNYAQQL
IQAAQAGNTDTTIEIDNGATLDGTYSLKEKNDSNGYHTTFSMTVKGKVTESNYDNVNADGKSKDDTEYESKMKDVAGVG
PKEYIETLNKEFVKAMGEEDGSPAGVEVVTGATHSTHSFINYAQQLVNAAEKGDITTEIVVDNIVTK

EFA0021

Seq ID 460

MDPQNIQTVKELRYALKQYKQTLATTIFDDTQGYISVAFKKEVIEKDGKNVQFLCFYPNYEVALYPVETLLAYLEKLEPQA
IVTIDKHGDNYSLSLFTIDETIDQGNNYQWLVISDKNSYERFFFTAQESTQEQAFKMLKELETDHDSLEVVHNWLCRQT
DRALFLGILKEDRTLKGAMTYCIGKAQEQSKDQSSAMVADEVVFSWIKYFLLLEKLPETKAVGKVTTKSETKPERKEVDE
QIDLFEV

EFA0044

Seq ID 461

MIILSGLFILGIGVGGYQLATLPKLIEMKQHKAIQNHFNKGNETTYQEDSENYILSLEDTEYRIKFSKNTPLKVVFTE
ILEPM

EFA0052

Seq ID 462

MKKIASTGLSILVATGVAGIGGNEVQAAEQAPKTPENSSTEQPTVKATQTTEQAITKQQQVTEKQAIVDQKQVADTAK
KEKDAIDQSVKDQAVVDONKDALDQSQQAVTDQQAVIDEAKKVDEATPSAIEKAKEQVATDTQAVDDQKQVDDQAQTDV
NQQAQVVEEKAKETNAKQVNDKQQAQVTAQKQAKLELAKNABAKAEKEQAQAEALANKQKEBAKADQKTKDD
QAVADQOSVVTTSQEKVADAKADTAQKADLTSKENALDKQAAATKQANTLDSKKEBKGHKGLNLPANFTFDYKKLTE
QEKQAMEKEALALNKVFPENQADAQKVTMINVNKPTKQKQMSDYVGLINDVREKLGLQKLKISNQAMKPAWDVAKYD
NPKEFDHVDNAINRAAKENGFKPEPPGNFYENLSMGRFTTQEGKVSMTDFEKAARNALVSMMLNDGHSYSHLDSLDANE
TNMAVSIISGLNDISAKIHIISYNQTKLVEANTYEEGTAPVFKSKETLQKEVATNQEKLATQAQARSQAQARSQAQALN
TAKTTQATAEKELS VHKATLANQAVATKSTTNYEEKVRQTAETNLQOTKDOLATINELIQNRAVVLEKAKTKVADAQA
IEQTSKVLKKEQEAQKAEENTLNSLKEVLDLAKENLNQKQVALKTSRSLARLENAQPTYEKAVNELNKAEAAVVQAKEA
YENSLKSLLEELKEQQAQVATLAYTQAQEDLSNAKLELQVQGIIRDLEAQQAQORRQEQALQEQVAKQORLEREAKQQLLV
ASATSADKTPGLQQLSFSKQKEQKPAQTLTHSEPRKTKQVAKAQESLPHTEGQKSIWLTIFGLFMAVGAIISFKNRRKNS

EFC0004

Seq ID 463

VILLVPASCGVNKSTEDSKKTNETKVEQLATLSAGTPVQSLDPATAVDQTSVTLLANVMEGLYRLDEKNQPPAIAAGQPKI
SNGGKTYTIVIRDGAKWADGTDITADDFVTAWQRVLDPKTASPNVELFAAIKNAKEISIGKQKQKETLGVKSKGNKTIIEIL
EPTPYFTDLLALTAYFFVQONAVKBYGKYGTTENIVTNGAFTLTDLNGVGISDKWTIAKNPKYWDKHHVAMEKIKFQV
VKDINTGINLYNDGQLDDAPVAGEYSKQLENNKDFIRELSATTMFLEVNQRNKKSTSNKHARQAINFAIDREAISNKILT
NGSIPAKGVVPSKLVYNPKTGKDFTNSSLVFLDKSKAKDSWEKAKKELKGTDLSDIMVNEEDLSKLGLEYLQNELQDTLD
GLKVSVTAVPATLQTERLNSGNFMIALSGWQADPADPVSFLANFESKSSLNHEGGYANKEYDKLLKNNSSKRLQELKDAEKI
ILEDAGVILLQIGNAKLRNQKISEMKVHSIGAKYDYKTMEIK

EFC0012

Seq ID 464

MKKIILSSLSFAVLVFGGGSITAFADDLGPTDPATPPITEPTDSSEPTNPTEPVDPAEPPVPTDPTPEPKTEPTTSPSE
EKTEPTTPTIDPGTPVEPTPEPSEPTPEPTPTPTPEPEKFTVPEQPKPTQPVPEKPAEPETPKTPROPTKPIDVVVT

PSGEIDKTNQSGAGTQPSIPIETSNLAETHVHPSETTPIITTEAGEEIVAVDKGVPLTKTPEGLKPISSSYKVLPSGNEVEKA
SDGKMKVLPHTGEKFTLLFVLSGSPFVLISGFFFFKKNKKKA

EFC0021

Seq ID 465

MVESKRTWKKEVHVESQVEERLRKNLTLKSREATKLQKKALKTTKKEFKLSKKNYKQSLKXHL/TLKTGKEPLSINHLLDK
KKFVEQKKRKAQKIAYKRTKKVDETRVVNQVKRETKKREATQKVRTTL/QEDTLNEAMTLYEKTQQAQKNMRTALKT
GKTVKNLSVKTAADTYGLGNRLFNFSRGRGFORTPKDFTLLKKQMLKQLNRNMRFFKAABAKKAEQIGLIRSFFNGQKTL
GKVAALILKNPISWVLLVLFVFLVLLSGVASSTQKPAIVQEEEDLTASWTYFTKLDAQHTDDNNLFYSNIDDLVLFYMNRY
DDFKLLDMDSTGTQNFETILSELNTALNGKKPDYQLKTMQSLTDKSSSYFIBEEQAKHYQEIKKELGYQTLDDLLSFPVK
TDALIVNRYGYDKSKKLTLYKGDVLIEDNQPFHSPMNGQIVSVPTTETLVIEKEKVARLTIRGVNTLRLLTKGMDVEEG
TFLGNTKNSVTTFQYKYYKKEKTDWFFVNPAPYFFPRVTTYTTLLGSAEFS PGASVEKRAQAVDYLSKKGYTKEGISAIL
GNFSVESGINPKRAEGDYLNPVGAHGNSWDEPSWLMGGPQIYGGRPFNILHRGLGLGQWTDADGGRRTLLLDYAKGK
NKKWYDLQLQDLDFIDGSDAPGSRTAADNVARSKVAATIPELTTYLTVEGNGPGDKLGERIQAAQNWTFPFSRSGTPIGGS
GSEVFAQYKKEKMQPLPTDRETAKGQWPGNAYAPGNCIYVFNRFALGKSIYPTMGNNANQWVHNYSQTPGATLESAPKKG
DAVIFTNGVAGSSTQYGHVATVEHVNSDGSFVISEMNVSGEYSMNWRVLKKEAGEYFMRLN

EFC0053

Seq ID 466

MTQVHPTLKSEIEQSIIEYSVKDDVSKNLTTFVFNQLMENQRTYIQAKEYERTENRQSQRNGYYERSFTTRVGTLELKVP
RTRDGHFSPTVFERYQRNEKALMASMLEMYVSGVSTRKVSKEVEELCGKSVSKSFVSSLTEQLEPMVNEWQNELLSEKNYP
YIMTDVLYIKVREENRVLSSKSCHIAIGITKGDREIIGFMIQSGESEETWTTFFEYLKERGLQGTTELVISDAHKGVLVAIR
KSFTNVSWQRQCVHFLRNIFTTIPKKNKSPREAVKGIFKFTDINLAREAKNRLIHDYIDQPKYSKACASLDDGPFEDAFQY
TVQGNSHNRLKSTNLIERLNQEVRRREKIIIRIFPNQTSANRLIGAVLMDLHDEWIYSSRKYINFDK

ARFC0021.1

Seq ID 467

psekgnggrvekrdsakstnhvnagryte

ARFC0021.2

Seq ID 468

enefklhkighlsfldrvhgsvvlgvrslrsikkkcnhypqtekqkqdkagqamrmrqqgivilgmclivlhs

ARF0324

Seq ID 469

lnkracesrsclsrnprgrnvgcdyckcrksac

ARF1627

Seq ID 470

vptlltnqlsvecqkhggqlfqlftkvghrllkqgptlpklkkc

ARF1650

Seq ID 471

krlqkklkksisqpirtekvkqllgi

CRF0097

Seq ID 472

mallpnvtskphgvaipvtgtfkpkkvttmptipenkfva

CRF0257

Seq ID 473

scafchvsvfgkyfrspatgtttipafavirkfnaltrsarasgeekaisadntsstgnr

CRF0635

Seq ID 474

gsivdnspdnainaigpaknktaktiv

CRF1152

Seq ID 475

sqggcygqlmllqyklfqnkhkldhkkddlcanwstslkqlpcfp

CRF1720

Seq ID 476

sfvqssycslrafsgkikfktipktvanaiednskvptlktppmpmtkiteainkfffflsklillsikifkplaaipn
ktklipetpggilrikalnfpkpkatantaappitktlctliavlastpifsp

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Organization
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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

Published:

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16 June 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **ENTEROCOCCUS ANTIGENS**

(57) Abstract: The present invention discloses isolated nucleic acid molecules encoding a hyperimmune serum reactive antigen or a fragment thereof as well as hyperimmune serum reactive antigens or fragments thereof from *E. faecalis*, methods for isolating such antigens and specific uses thereof.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/005664

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/315 C12N15/31 C12Q1/68 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, Sequence Search, PAJ, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE Geneseq 'Online! 19 June 2003 (2003-06-19), "Protein encoded by Prokaryotic essential gene #14577." XP002303524 retrieved from EBI accession no. GSN:ABU29050 Database accession no. ABU29050 The sequence is 100% identical with SEQ ID NO: 171 & WO 02/077183 A (MALONE CHERYL ; OHLSEN KARI L (US); WALL DANIEL (US); XU H HOWARD (US)) 3 October 2002 (2002-10-03)</p> <p style="text-align: center;">----- -/--</p>	<p>1,2, 5-28, 30-37</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

A document member of the same patent family

Date of the actual completion of the international search

2 November 2004

Date of mailing of the international search report

28.01.05

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP2004/005664

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE UniProt 'Online! 1 June 2003 (2003-06-01), "PTS system, mannose-specific IIAB components. VLFLVDLWGG TPFNQANSL EDHKDKWAIV AGMNLPMVIE AYASRFSMES AQEIATHILE" XP002303525 retrieved from EBI accession no. UNIPROT:Q839X9 Database accession no. Q839X9 The sequence is 100% identical with SEQ ID NO: 171</p> <p>-----</p>	1,2, 5-28, 30-37
X	<p>DATABASE Geneseq 'Online! 22 April 2004 (2004-04-22), "Enterococcus faecalis polypeptide #1547." XP002303526 retrieved from EBI accession no. GSN:ADH87067 Database accession no. ADH87067 100% identity with SEQ ID NO: 171 & US 6 617 156 B1 (DOUCETTE-STAMM LYNN A ET AL) 9 September 2003 (2003-09-09)</p> <p>-----</p>	1,2, 5-28, 30-37
X	<p>DATABASE Geneseq 'Online! 1 January 2004 (2004-01-01), "E. faecium protein sequence SEQ ID 6586." XP002303527 retrieved from EBI accession no. GSN:ADC96959 Database accession no. ADC96959 The sequence has 86% identity with SEQ ID NO: 171 & US 6 583 275 B1 (DOUCETTE-STAMM LYNN A ET AL) 24 June 2003 (2003-06-24)</p> <p>-----</p>	1,2, 5-28, 30-37
X	<p>DATABASE Geneseq 'Online! 18 December 2002 (2002-12-18), "Enterococcus faecalis contig sequence #464." XP002303528 retrieved from EBI accession no. GSN:ABS99196 Database accession no. ABS99196 The sequence is 100% identical with SEQ ID NO: 1</p> <p>-----</p> <p style="text-align: center;">-/--</p>	1,2,5-10

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/005664

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE UniProt 'Online! 1 October 2001 (2001-10-01), "Mannose-specific phosphotransferase system component IIAB. VLFLVDLWGG TPFNQANS LF EEHKDKWAIV AGMNLPMVIE AYGARLSMES AHEIAASIIS" XP002303529 retrieved from EBI accession no. UNIPROT:Q97TN2 Database accession no. Q97TN2 -----</p>	
A	<p>HENICS T ET AL: "Small-fragment genomic libraries for the display of putative epitopes from clinically significant pathogens" BIOTECHNIQUES, EATON PUBLISHING, NATICK, US, vol. 35, no. 1, July 2003 (2003-07), pages 196-200,202,20, XP002293668 ISSN: 0736-6205 -----</p>	
T	<p>MEINKE ANDREAS ET AL: "Bacterial genomes pave the way to novel vaccines" CURRENT OPINION IN MICROBIOLOGY, vol. 7, no. 3, June 2004 (2004-06), pages 314-320, XP002303523 ISSN: 1369-5274 -----</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2004/005664

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 29
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 2, 5-28, 30-37 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims 1, 2, 5-28, 30-37 (all partially)

Claims relating to a nucleotide sequence and the corresponding proteins sequence having SEQ ID NOs: 1 and 171, the use of said sequences and antibodies directed against said protein

Inventions 2 to 166: Claims 1, 2, 5-28, 30-37 (all partially)

Claims relating to the nucleotide sequences and the corresponding proteins sequence having SEQ ID NOs: 2 to 170 and 172 to 340 (with the exception of SEQ ID NOs: 19, 28, 78, 93 respectively 189, 198, 248 and 263), the use of said sequences and antibodies directed against said protein

Inventions 167-218: Claims 3, 5-28, 30-37 (all partially)

Claims relating to a nucleotide sequences and the corresponding proteins sequence having SEQ ID NOs: 373 to 424 and 425 to 476 respectively, the use of said sequences and antibodies directed against said protein

4. claims: Inventions 219-220: Claims 4-28, 30-37 (all partially)

Claims relating to a nucleotide sequences and the corresponding proteins sequence having SEQ ID NOs: 90 and 147 and 260 and 317 respectively, the use of said sequences and antibodies directed against said protein

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 29

Claim 29 relates to an agonist which lacks any meaningful technical characterisation. Therefore a search for accordingly defined compounds was not possible

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2004/005664

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02077183	A	03-10-2002	US 2002061569 A1 23-05-2002
		US 02077183 A2 03-10-2002	
		US 2004029129 A1 12-02-2004	
US 6617156	B1	09-09-2003	NONE
US 6583275	B1	24-06-2003	NONE